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FORM PTO-1390 U S Department of Commerce Patent and Trademark Office	Attorney's Docket No. 2328-117
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U.S. Application No. (if known, see 37 CFR 1 5) 09/529967

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FI98/00873	11 November 1998	14 November 1997

TITLE OF INVENTION TETRACYCLINE ASSAY METHOD

APPLICANT(S) FOR DO/EO/US Matti KORPELA, Matti KARP, and Jussi KURITTU

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [XX] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [XX] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [XX] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [XX] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [XX] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [XX] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [XX] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [XX] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [XX] A change of power of attorney and/or address letter.
16. [XX] Other items or information:
    Small Entity Statement

09529967 042400

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U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/529967	INTERNATIONAL APPLICATION NO PCT/FI/00873	ATTORNEY DOCKET NO. 2328-117

	CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted. **Basic National Fee (37 CFR 1.492)(a)(1)-(5):** Search Report has been prepared by the EPO or JPO $ 840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) $ 670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $ 690.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $ 970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $ 96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 970.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20 -20 =		X $18.00	$	
Independent Claims	3 - 3 =		X $78.00	$	
Multiple dependent claim(s) (if applicable)			+ $260.00	$	

TOTAL OF ABOVE CALCULATIONS =	$ 970.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).	$ 485.00	
SUBTOTAL =	$ 485.00	
Processing fee of $130.00 for furnishing the English translation later [ ] 20 [ ] 30 than months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$ 485.00	
	Amount to be refunded	$
	charged	$

a. [XX] A check in the amount of $ 485.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. 02-2135 in the amount of $________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2135. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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Registration Number

Dated: 24 April 2000

09/529967

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Matti KORPELA et al.	)	U.S. National Phase,
	)	PCT/FI98/00873
Serial No. (to be assigned)	)	
	)	Intl. Filing Date:
Filed: 24 April 2000	)	11 November 1998
	)	
For: TETRACYCLINE ASSAY METHOD	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-identified U.S. National Phase of PCT/FI98/00873, filed concurrently herewith, please enter the following amendments thereto:

IN THE CLAIMS:

Please amend the claims as follows:

Claim 3, line 1, delete "or 2".

Claim 5, line 1, delete "or 2".

Claim 7, line 1, change "any of the claims 1-6" to -- claim 1 --.

Claim 8, line 1, change "any of the claims 1-6" to -- claim 1 --.

Claim 9, line 1, change "any of the claims 1-8" to -- claim 1 --.

Claim 10, line 1, change "any of the claims 1-9" to -- claim 1 --.

Claim 13, line 1, delete "or 12,".

Please add the following new claims:

-- 16. The method according to claim 2 characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO:3), tetracycline repressor (TetR) (SEQ ID NO:11) and tetracycline promotor (TetA) (SEQ ID NO:9) from *Tn*10. --

-- 17. The method according to claim 16 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO:3). --

-- 18. The method according to claim 2 characterized in that

- the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO:1), tetracycline repressor (TetR) (SEQ ID NO:11) and tetracycline promoter (TetA) (SEQ ID NO:9) from *Tn*10, and that

- D-luciferin is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells. --

-- 19. The method according to claim 18 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO:1). --

-- 20. The cell according to claim 12 characterized in that it is in dried form, e.g., in lyophilized form. --

REMARKS

The claims have been amended to delete multiple dependencies and to bring them more into conformance with U.S. practice. No new matter has been added by the above amendments, and their entry is therefore requested.

Respectfully submitted,

By Jeffrey L. Ihnen

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Dated: 24 April 2000

SMALL ENTITY DECLARATION

APPLICANT OR PATENTEE KORPELA, Matti, KARP, Matti and KURITTU, Jussi

SERIAL NO. ____________ ☐ PATENT NO ____________ DOCKET NO. ____________

(Check one of blocks 1 or 2)

1. ☐ FILED OR ISSUED ____________

2. ☐ SUBMITTED HEREWITH ____________

FOR "Tetracycline assay method"
(Insert Title)

I(we) hereby declare that I(we) am(are) entitled to the benefit of small entity status with respect to the above-identified application or patent for purposes of paying reduced fees under 35 USC 41(a) & (b) to the U.S. Patent and Trademark Office.

[X] A. INDEPENDENT INVENTOR
I(we) qualify as (an) independent inventor(s) as defined in 37 CFR 1.9(c).

☐ B. INDIVIDUAL NON-INVENTOR
I would qualify as an independent inventor as defined in 37 CFR 1.9(c) if I had made the invention.

☐ C. SMALL BUSINESS CONCERN
I am ☐ THE OWNER ☐ AN OFFICIAL of the small business concern identified below and am empowered to act on behalf of the concern. The concern qualifies under 37 CFR 1.9(d) and 13 CFR 121.1301-1305. Rights under contract or law have been conveyed to and remain with the concern and are exclusive unless a checkmark is placed here ☐. All other rights belong to small entities as defined in 37 CFR 1.9.

☐ D. NON-PROFIT ORGANIZATION
I am an official am empowered to act on behalf of the non-profit organization identified below. The organization qualifies under 37 CFR 1.9(e), sub section: ☐ (1) ☐ (2) ☐ (3) ☐ (4). Rights under contract or law have been conveyed to and remain with the organization and are exclusive unless a checkmark is placed here ☐. All other rights belong to small entities as defined in 37 CFR 1.9.

I(we) acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I(we) declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

A. INDEPENDENT INVENTOR(S)

Name	Signature	Date
KORPELA, Matti	Matti Korpela	18.4.2000
KARP, Matti	Matti Karp	17.4.2000
KURITTU, Jussi	Jussi Kurittu	19.4.2000

Tetracycline assay method.

FIELD OF THE INVENTION

This invention relates to a method for the determination of a tetracycline in a sample. The invention also concerns recombinant prokaryotic cells capable of emitting light in response to the existence of a tetracycline in a sample. Furthermore, the invention relates to novel DNA vectors useful for the construction of said prokaryotic cells.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

Whole cells can be used in methods based on the use of living cells or organisms as sensor tools of detection. Many of these methods utilize bacterial or yeast cells. Prokaryotic organisms and especially *Escherichia coli* bacterium are very well characterized and maps of genes and their sequences at nucleotide level are known. Therefore the behavior of the whole cell sensor can be better understood. Because of this fact it is also possible to develop analyte or group specific sensors utilizing different regulatory regions of genomes and also various microbial strains.

Whole cells can be utilized in biosensors which are devices consisting of 1) a sensor, 2) a recording unit and 3) a possible connector such as fiber optic guide between 1 and 2. The recording unit has several choices of what is the physical background of the measurement. It can be change in heat, conductance, color reaction, changes in fluorescent properties, emission of endogenous light from the sensor cells etc.

Antibiotics used as medicines against microbial invasion are detected from body fluids in order to study the dosage and penetration of the medicine. Often the effective therapeutic range of the antibiotic is rather narrow and the risks of overdosage might be too big. It is also important to measure the presence or concentration of antibiotics from meat and milk due to syndrome of allergic people. In the course of cheese production milk used as starting material should not contain antibiotics due to the fact that cheesemaking bacteria are not able to work on contaminated milk.

Conventional tests for the measurement of toxic substances such as antimicrobial agents (antibiotics) are based on the inhibition of growth. Growth inhibition can be followed by monitoring the zone where the growth of microbes is inhibited on a nutrient agar plate around a disk onto which an antibiotic dilution was pipetted. Typical examples of agar diffusion tests are cylindrical, hole or disk methods. The difference in these tests is only restricted in the way the sample is applied on the agar and also the way the bacteria in the test is used. Another means is to follow the metabolism of the test organisms by estimating the intensity of a color reaction which is affected by the inhibitory antibiotic present and comparing it to the uninhibited control (e.g. the commercial products: Delvo Test™, Brilliant black-reduction test, Charm Farm Test, Charm AIM-96 and Valio T101-test). Since microbiological methods utilize bacteria or their spores it is the sensitivity of the test bacteria which is of utmost importance. Thus far one had to make compromises in the choice of a suitable test strain since great sensitivity against antimicrobial agents and other characteristics needed for the test strain have not been common features for the same strain of bacteria. A major drawback when using microbes in antibiotic residue tests is slow and unsensitive performance. Since in these methods one always controls in a way or other the growth of the tester strain one cannot imagine

the test to be performed in an hour. This is due to the fact that the growth of the microbe is a slow phenomenon even at its fastest mode. Also in many cases microbes are in spores or freeze-dried, the regeneration of which makes the tests even more slow to perform.

OBJECT AND SUMMARY OF THE INVENTION

The object of the invention is to provide a novel method of determining a tertracycline in a sample where said method is rapid and selective for tetracyclines, i.e. the method is able to distinguish tetracyclines from other antimicrobial agents.

According to one aspect of the invention a method for the determination of a tetracycline in a sample is provided, wherein the method is characterized in that

- the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
- detecting the luminescense emitted from the cells, and
- comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
- wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.

According to another aspect, the invention concerns a recombinant prokaryotic cell which encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme, tetracycline repressor and tetracycline promoter.

According to yet another aspect, the invention concerns a plasmid which comprises either

- the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10, or
- the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a shows schematically the method according to this invention, where cells cloned with the plasmid pTetLux1 (SEQ ID NO: 3) are used.

Figure 1b shows schematically the method according to this invention, where cells cloned with the plasmid pTetLuc1 (SEQ ID NO: 1) are used.

Figure 1c shows schematically the production of the luciferase enzyme,

Figure 2 shows the plasmid pTetLux1 (SEQ ID NO: 3).

Figure 3 shows the plasmid pTetLuc1 (SEQ ID NO: 1).

Figure 4a shows the production of light (induction factor) versus concentration of tetracycline in samples for three different tetracyclines,

Figure 4b shows the production of light (induction factor) versus concentration of tetracycline in samples for further four different tetracyclines.

Figure 5 shows the effect of magnesium ions on the sensitivity of the method according to the invention.

Figure 6 illustrates possibilities of changing the assay window for the method of the invention by adjusting magnesium ion concentration and pH.

Figure 7 shows the induction factor versus tetracycline concentration when using freeze-dried *E. coli* in the determination of tetracycline.

Figure 8 shows a comparison of the assays based on using cells with the plasmid pTetLuc1 (SEQ ID NO: 1) and with the plasmid pTetLux1 (SEQ ID NO: 3).

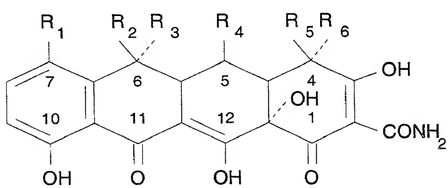
Figure 9 shows induction factors versus antibiotic concentrations of a pig serum sample (cells *E. coli* K12, pTetLux1).

Figure 10 shows the effect of EDTA in a milk sample assay, and

Figure 11 shows the light emission versus time for an assay according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

The term "tetracycline" shall be understood to include any compound covered by the general structure formula



and particularly the specific commercially available compounds listed in the table below.

GENERIC NAME	R_1	R_2	R_3	R_4	R_5	R_6
Chlorotetracycline	Cl	OH	CH_3	H	H	$N(CH_3)_2$
Demethylcholorotetracycline	Cl	OH	H	H	H	$N(CH_3)_2$
Doxycycline	H	H	CH_3	OH	H	$N(CH_3)_2$
Methacycline	H	CH_3	H	OH	H	$N(CH_3)_2$
Minocycline	$N(CH_3)_2$	H	H	H	H	$N(CH_3)_2$
Oxytetracycline	H	OH	CH_3	OH	H	$N(CH_3)_2$
Tetracycline	H	OH	CH_3	H	H	$N(CH_3)_2$

Furthermore, the term "tetracycline" shall be understood to cover the metabolic and other reformulation/decomposition products thereof.

The cells useful in the method of the invention are preferably *Escherichia coli*, which are stored in dried form, e.g. in lyophilized form before their use in the method according to the invention. Also freshly cultivated cells can be used.

According to a preferred embodiment, the DNA vector including a nucleotide sequence encoding a light producing enzyme is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from transposon *Tn*10. Particularly preferable is the plasmid pTetLux1 (SEQ ID NO: 3).

According to another preferred embodiment, the DNA vector including a nucleotide sequence encoding a light producing enzyme is a plasmid containing the insect

luciferase gene, tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10. In this case the substrate for insect luciferase reaction, D-luciferin, is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells. The plasmid is preferably pTetLuc1 (SEQ ID NO: 1).

The method according to this invention is useful for the determination of tetracycline in various kinds of samples. As examples can be mentioned milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the like.

The luminescence of the cells is preferably measured using an X-ray or polaroid film, a CCD-camera (Charge Coupled Device), a liquid scintillation counter or, most preferably, a luminometer.

The sensitivity of this analysis method with respect to the tetracycline can be controlled by increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, in the mixture of the sample and the cells, by adjusting the pH or by combined adjusting of the divalent metal ion concentration and the pH. Increasing concentration of magnesium ions decreases the sensitivity and vice versa. Increasing pH will also cause a decreasing sensitivity. The sensitivity of the analysis with respect to the tetracycline can be increased by the use of cells which are especially antibiotic sensitive mutant strains. Chelating agents such as EDTA can be added to further sensitize the sensor system for tetracyclines.

Figures 1 show a schematic representation of a method based on specific detection of the presence of tetracyclines using microbial cells cloned with either the plasmid pTetLux1 (SEQ ID NO: 3) (Figure 1a) or with the plasmid pTetLuc1 (SEQ ID

NO: 1) (Figure 1b). The figures show that cells containing either of the plasmids can be triggered to produce light by adding a chemical agent (a tetracycline). Light production is a consequence of tetracycline responsive promoter activation due to removal of the tet-repressor protein (SEQ ID NO: 11) leading to the production of luciferase specific mRNA and luciferase protein (SEQ ID NO: 2, 4-8) itself. The principle is demonstrated in Figure 1c. In case of the usage of full length bacterial luciferase operon (SEQ ID NO: 3) containing *lux*C, *lux*D, *lux*A, *lux*B and *lux*E genes (SEQ ID NO: 3) (Figure 1a), one is able to get light emission without addition of any substance. In case of insect (e.g. firefly) luciferase (SEQ ID NO: 2) (Figure 1b), light is emitted only after the addition of D-luciferin. It should be noticed that the triggering of luciferase synthesis and light production commences immediately when the cells are introduced to the inducer molecules (tetracyclines). Therefore there is no need to use dividing cells and hence there is no need to use long cultivation of microbial cells such as the case is with conventional methods. Therefore, if needed, one can get results in minutes rather than in hours or days which is the case when conventional methods are used.

Figure 2a shows the plasmid pTetLux1 (SEQ ID NO: 3), in which the production of bacterial luciferase (SEQ ID NO: 4-8) of *Photorhabdus luminescens* (formerly *Xenorhabdus luminescens*; the lux-operon structure and the full-length nucleotide sequence of *P. luminescens* was published in Szittner, R. and Meighen, E. (1990) J. Biol. Chem. 265, 16581-16587) can be switched on by the addition of a chemical agent belonging to the tetracycline family of antimicrobial agents in a cloned *E. coli* bacterium. SEQ ID NO: 3 shows the nucleotide sequence of the plasmid pTetLux1. This plasmid construct is devised to contain the five genes from *P. luminescens* luciferase operon necessary for the light production without any additions of substrates, i.e. cells cloned with such a construct produce substrates endogenously. By incubating *E. coli* cells containing this plasmid (or any other microbial strain

whereto similar regulation/reporter gene system is incorporated containing the necessary secondary regulatory sequences in the constructs such as correct ribosome binding region, transcriptional termination etc.) in the presence of very small amounts of tetracyclines one is able to obtain light production the intensity of which is proportional to the concentration of tetracycline used.

Any *E. coli* mutant strain and especially those strains having a mutation in the export/import machinery of the membranes or otherwise leaky character making it possible for large molecules to easily penetrate inside the cell would be beneficial to use in the method described in this invention. Also other gram-negative bacteria such as strains belonging to genus *Salmonella, Shigella, Enterobacter, Citrobacter, Klebsiella, Erwinia, Pseudomonas, Serratia* as well as gram-positive organisms such as those belonging to genus *Bacillus* (especially *B. subtilis, B. licheniformis, B. pumilus, B. globigii, B. natto, B. amyloliquefaciens* as well as *B. niger, B. brevis, B. megaterium*), *Streptomyces, Lactobacillus* (especially *L. lactis, L. casei*) and *Streptococcus* (especially *S. thermophilus, S. cremoris, S. agalactiae*) come into question. Especially asporogenic strains of *Bacilli* or *Lactobacilli* are suitable.

Figure 3 shows the plasmid pTetLuc1 (SEQ ID NO: 1), in which the production of firefly luciferase (SEQ ID NO: 2) of *Photinus pyralis* (The gene encoding firefly luciferase was originally cloned and sequenced in the middle of the 1980's by DeWet, J. et al. (1987) Mol. Cell. Biol. 7, 725-737) can be switched on by the addition of a chemical agent belonging to the tetracycline family of antimicrobial agents in a cloned *E. coli* bacterium. SEQ ID NO: 1 shows the nucleotide sequence of this plasmid. By incubating *E. coli* cells containing this plasmid (or any other microbial strain whereto similar regulation/reporter gene system is incorporated containing the necessary secondary regulatory sequences in the constructs such as correct ribosome binding region, transcriptional termination etc.) in the presence of

very small amounts of tetracyclines one is able to obtain light production by the addition of D-luciferin, which is the substrate of firefly luciferase. The intensity of light emission is proportional to the concentration of tetracycline used.

Figures 4a and 4b shows the effect of altogether seven different tetracyclines on the production of light as a function of concentration of each tetracycline. As controls different non-tetracycline antibiotics were included in this study to show that the sensor strain is specific for the tetracyclines. The luminescense was emitted from *E. coli* containing the plasmid pTetLux1 (SEQ ID NO: 3). The detection was made after an incubation of 90 min. All tetracyclines tested behaved in a very similar manner and induction efficiencies were at the same antibiotic concentration area. This makes this sensor even more attractive for analytical use for the determination of the tetracycline group of antibiotics.

It should be noted that the accumulation of various tetracyclines into microbial cells is very strongly affected by the extracellular concentration of Mg^{2+} ions. Figure 5 shows the effect of increasing concentrations of Mg^{2+} ions on the behavior of *E. coli* cells containing the plasmid pTetLux1 (SEQ ID NO: 3). As can be seen the tetracycline response curve is shifted to the right as a function of increasing concentrations of added Mg^{2+} ions. Thus by increasing the Mg^{2+} ion concentration one is able to decrease the sensitivity of the tetracycline sensor described in this invention. This fact is of great importance in cases where one does not need a high sensitivity of the measurement and where the approximate concentration of the ion is roughly constant and known such as in milk, serum and plasma.

The sensitivity can be increased by removing magnesium ions from the assay mixture e.g. by adding a chelating agent forming a complex with magnesium.

Figure 6 shows the possibilities to change the assay window for tetracyclines by adjusting the magnesium ion concentration and by combined adjustment of the magnesium ion concentration and pH.

The sensitivity of the assay can be increased by the use of cells which are especially antibiotic sensitive mutant strains. Hundreds of specific mutations for bacteria are known with which it is possible to study the activity of specific reactions. For instance trace amounts of antibiotics cause visible changes in the metabolism or in the cell membranes of antibiotic sensitive bacterial mutants. Mutations in cell wall structural components or biosynthetic enzymes as well as in transport and efflux proteins such as porins might have an effect on the behavior of each sensor. Using these kinds of mutations one is able to develop tests measuring residual antibiotics from biological material very sensitively. It is also rather simple to transfer new characteristics into bacterial cells by genetic engineering techniques. This phenomenon broadens the applicability of these organisms in tests utilizing whole cell sensor.

Measurement of light emission can be done by using X-ray or polaroid film, using a liquid scintillation counter, a CCD-camera or a luminometer. The CCD-camera is an instrument which is capable of detecting very low levels of light. In the applications of this invention such kind of a device could be used for the detection of tetracycline residues in food material such as vegetables or meat. The detection of light emission could be directly monitored from the surface of the food material sprayed with engineered luminescent bacteria. Either chemiluminescent (such as peroxidase - luminol) or bioluminescent (such as luciferase - luciferin) reactions can be utilized. The luminometric method is performed with the aid of genes encoding either bacterial or beetle luciferases such as those described in the Figures 2 and 4. Several luminescent bacterial species such as *V. harveyi*, *V. fischeri*, *P. leiognathi*,

P. phosphoreum, *Xenorhabdus luminescens* etc. exist. Luminescent beetles are for example *Luciola mingrelica*, *Photinus pyralis*, *Pyrophorus plagiophthalamus*, *Lampyris noctiluca, Pholas dactylus,* etc. Also several eukaryotic species in the sea which luminesce, such as marine ostracod *Vargula hilgendorfii*, jellyfish *Aequorea victoria*, batrachoidid fish *Porichtys notatus*, pempherid fish *Parapriacanthus ransonneti* etc. exist. Fluorescent reporter proteins such as green fluorescent protein (GFP) or any of its variants could be used in the methods described in this invention (Li, X. et al. (1997) J. Biol. Chem. 272, 28545-28549).

In this invention high detection sensitivity of the luminescent enzyme labels inside a living cell associated with tetracycline-specific induction of label synthesis is based on the use of optimal concentration of all the reactants inside the cell including the necessary cofactors and accessory enzymes. All luciferase genes from these organisms would presumably work in a similar manner as the two examples shown in this invention. These systems together with enhancers and modulators (wavelength, emission kinetics etc.) of light emission has been described in more detail in Campbell, A. "Chemiluminescence; principles and applications in biology and medicine", Weinheim; Deerfield Beach, Fl.; VCH; Chichester: Horwood, 1988.

Peroxidases or oxidases can be used together with compounds such as luminol or acridines (for instance lucigenin) to yield luminescent signals suitable for a detection system described here. Enzymatically generated chemiluminescence offers great sensitivity and rapid detection, too, in assays described in this invention. Thermally stable dioxetanes (such as AMPPD and Lumigen PPD) can be enzymatically (such as alkaline phosphatase or β-galactosidase) triggered to produce chemiluminescence (Schaap, A.P. et al. (1989) Clin. Chem. 35, 1863-1864). The only difference to the luciferase enzymes would be that these enzymes are capable

of cleaving a man-made substrate which gives light emission (chemiluminescence) and the luciferases cleave natural substrates to produce light (bioluminescence).

Tetracycline-controlled expression systems are developed to express heterologous proteins in procaryotic and eucaryotic cells for the purpose of production under a tight control of tet-regulatory system (Skerra, A. (1994) Gene 151, 131-135; Gossen, M. and Bujard, H. (1995) US Patent 5,464,758 ; Lutz, R. and Bujard, H. (1997) Nucleic Acids Res. 25, 1203-1210).

A method to study various tetracyclines and their mode of action was developed by Chopra et al. (Chopra, I. et al. (1990) Antimicrob. Agents Chemother. 34, 111-116) The assay system developed in this study was based on expression of β-galactosidase gene inserted under the control of tetA-gene. The method resulted in less sensitive detection of tetracyclines compared to the invention described here. However in order to obtain maximum sensitivities Chopra et al. showed that it was necessary to add cyclic AMP (cAMP) to the medium which is an extremely expensive molecule to be used in routine applications. Furthermore, the method described by Chopra et al. contains a cell disruption stage by sonication in order to assay for the reporter gene activity, β-galactosidase, which step is not practical. Instead, the method described in this invention does not contain any cell disruption. The activity of luciferase can be measured directly from living cells in real-time and in the case of pTetLux1 (SEQ ID NO: 3) there is no need of addition of any substrates. Therefore, promoter activation due to the presense/absense of tetracycline can be monitored continuously.

EXPERIMENTS

As cloning hosts and in antibiotic residue measurements various *E. coli* MC1061 (*c*I+, *ara*D139, Δ(*ara-leu*)7696, *lac*X74, *gal*U, *gal*K, *hsr*, *hsm*, *str*A) (Casadaban,

M.J. and Cohen, S.N. (1980) J. Mol. Biol. 138, 179-207), BW322 (CGSC, *rfa*210::Tn10, *thi*-1, *rel*A1, *spo*T1, *pyr*E) and K-12 (M72 Sm^R *lac*Zm-Δ*biouvr*B, *trp*EA2, *N*am7*N*am53*c*I857 HI) (Remaut, E. et al. (1981) Gene 15, 81-93) can be used. Especially the strain LH530 (Hirvas, L. et al. (1997) Microbiology 143, 73-81) which has a decreased rate of lipid A biosynthesis. It has proven to be hypersusceptible to many different antibiotics.

Cells were grown on appropriate minimal agar-plates and were kept maximally one month at +4 °C after which new plates were stroked. The strains were kept also in 15% glycerol at -70 °C, where from growth was started through minimal plates. The cells were first cultivated in 5 ml of 2xTY medium (16 g Bacto tryptone, 8 g Yeast extract, 8 g NaCl, H_2O ad 1 l, pH 7.4, with appropriate antibiotic) 10 h at 30 °C in a shaker after which the cultivation was transferred to a bigger volume for 10 h with same medium.

Construction of tetracycline-responsive sensor plasmids:

To construct a recombinant DNA vector carrying luciferase genes under the control of a tetracycline responsive elements two new vectors were created. In the first one modified firefly luciferase gene (SEQ ID NO: 1) from vector pBLuc* (Bonin, A.L. et al. (1994) Gene 141, 75-77) was excised by using restriction enzymes *Xba*I and *Hin*DIII and the 1.7 kb fragment was isolated from LGT-agarose gel and purified using Qiagen gel extraction kit. This DNA-fragment containing the entire *Photinus pyralis* luciferase gene (SEQ ID NO: 1) was ligated using T4-DNA-ligase enzyme to vector pASK75 (Skerra, A. (1994) Gene 151, 131-135) which was previously restricted with the same restriction enzymes *Xba*I and *Hin*DIII and calf intestinal phosphatase treated to remove the protruding phosphate groups in order to prevent self-ligation. The resulting ligation mixture was incubated 3 hours at room temperature after which one μl of the mixture was electroporated according to

Dower *et al.* (Dower, W.J. et al. (1988) Nucleic Acids Res. 16, 6126-6144) into electrocompetent *E. coli* MC1061 cells. A plasmid was extracted from one of the colonies obtained and checked for the estimated structure by appropriate restriction enzyme digestions and agarose gel electrophoretic techniques. The plasmid obtained was named as pTetLuc1 (SEQ ID NO: 1).

The plasmid containing the luxCDABE genes (SEQ ID NO: 3) of *Photorhabdus luminescens* under the control of tetracycline responsive element was created as follows: Plasmid pASK75 was cut with restriction enzyme *Eco*RI and CIP-treated. The linearized plasmid was separated on a LGT-agarose gel electrophoresis and the agarose was removed by using the Qiagen kit. The lux operon was excised with EcoRI from plasmid pCGLS-11 (Frackman, S. et al. (1990) J. Bacteriol. 172, 5767-5773), gel purified as above and ligated to pASK75 by using T4-DNA-ligase at 16 °C overnight. The ligation mixture was electroporated into *E. coli* MC1061 cells as described above and correct transformants were screened for their ability to produce light (as measured with a BioOrbit 1250 manual luminometer) which production was increased in the presence of 1 μg/ml of tetracycline-HCl. The plasmid was further verified by restriction enzyme digestions and the correct structure was named as pTetLux1 (SEQ ID NO: 3). All the DNA-manipulations were performed according to Sambrook *et al.*, "Molecular Cloning: A laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

The vector pASK75 was utilized in the construction of tet-sensor plasmids shown in this invention. The vector pASK75 was originally developed for protein production and purification purposes. It contains a signal sequence for secretion of the recombinant protein into the periplasmic space of *E. coli*. Also a C-terminal fusion between a purification tail, the Strept-tag, was incorporated into the vector to facilitate purification of recombinant protein using streptavidin affinity agarose gel

chromatography. The element controlling recombinant gene expression in the vector is tetA promoter/operator system that allows efficient regulation of the expression, which in Skerra's paper was described for the production and one-step purification of a murine single-chain antibody fragment. The tetA promoter/operator (SEQ ID NO: 9) is controlled by tetR-repressor (SEQ ID NO: 9) which is produced by the corresponding gene (SEQ ID NO: 9). Some of the above mentioned elements were eliminated from the present plasmids due to unnecessary features with respect to this invention.

Transfer of the tetracycline sensor vectors to the antibiotic sensitive *E. coli* strain:

Either pTetLux1 (SEQ ID NO: 3) or pTetLuc1 (SEQ ID NO: 1) was transformed into *E. coli* LH530 cells by electroporation as described above. The transformed cells were restreaked on agar plates and kept maximally for 2 weeks at +4 °C after which a new plate was streaked.

Use of the manipulated *E. coli* in tetracycline determination methods:

Example 1

Freeze-dried *E. coli* K-12/pTetLux1 were reconstituted with 1.0 ml of L-broth and bacteria were diluted 1:10 with 25 mM MES buffer in M9 minimal medium, pH 6.0. 190 µl bacterial suspension was added to microtiter plate wells containing 10 µl of tetracycline dilutions. The plate was incubated 90 minutes at 37 °C after which the plate was measured with Labsystems Luminoskan luminometer. As seen from Figure 7 the sensitivity of the assay of tetracycline is very high and comparable to that of fresh cells.

Example 2

Two different types of sensor DNA vector construct were compared. Strains *E. coli* K-12/pTetLux1 and *E. coli* K-12/pTetLuc1 were cultivated in L-broth media until optical density measured at 600 nm (OD600) was 1.5. The cells were diluted 1 to 50 with 25 mM MES-buffer in M9 minimal medium, pH 6.0 (Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory, Cold Spring Harbor) and 190 µl was added to microtitration plate wells and 10 µl of sample dilution of tetracycline was added. After a 60 min incubation at 37 °C the light emission was measured using a Labsystems Luminoskan luminometer. Figure 8 shows the bioluminescence dose response curve as a function of tetracycline added. As seen from the figure both systems (bacterial and insect luciferase) give roughly equal sensitivity of tetracycline detection.

One is able to use different luciferases instead of bacterial luciferase (SEQ ID NO: 4-8) from *P. luminescens* without losing sensitivity or other performance of the test. Figure 8 shows an analogous measurement to the one in Figure 4b. In the plasmid used in this test (pTetLuc1) the bacterial luciferase was compensated with firefly luciferase (SEQ ID NO: 2) as described in Figure 3. The test was done essentially as with bacterial luciferase except that after the cells had been incubated with or without tetracycline 10 minutes at 37 °C the cells were measured for light production after 15 minutes incubation time at 37 °C by adding 100 µl of solution containing 1 mM D-luciferin, in 0.1 M Na-citrate buffer, pH 5.0. Thereafter light production was measured using a manual luminometer 1250 (LKB-Wallac, Turku, Finland). As can be seen from Figure 8 sensitivity of the method to detect tetracycline hydrochloride is extremely high and comparable to the detection made with bacterial luciferase.

Example 3

A lipemic pig serum was spiked at different concentrations of tetracycline, chlorotetracycline and oxytetracycline. Fresh *E. coli* K-12/pTetLux1 were diluted 1:50 with 25 mM MES buffer in M9 minimal medium, pH 6.0. 100 µl bacterial suspension was added to microtiter plate wells containing 100 µl of pig serum spiked with different tetracyclines. The plate was incubated 90 minutes at 37 °C after which the plate was measured with Labsystems Luminoskan luminometer. As seen from Figure 9 the sensitivity of the assay of different tetracyclines in pig serum matrix is very high.

Example 4

Tetracyclines will form chelate complexes with Ca^{2+} and Mg^{2+} in samples (e.g. milk), and loose their antimicrobial and induction activity in our assay system. Tetracyclines can be displaced from cation chelates by using strong chelating agents such as EDTA. Figure 10 shows the determination of tetracycline from a milk sample, which is spiked with different concentrations of tetracycline. Different amounts of EDTA were added to milk samples and this kind of displacement of cation-tetracycline complex clearly improved the sensitivity of the assay. In the assay we used freeze-dried *E. coli* K12/pTetLux1 that were reconstituted with L-broth 10 minutes in room temperature before the assay.

Example 5

Figure 11 shows the kinetics of bacterial bioluminescence after exposure of *E. coli* K-12/pTetLux1 to different dilutions of tetracycline antibiotics. The specific induction of tetracycline is very fast and specific light emission is seen already at the 10 minutes measuring point in the assay.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: KORPELA, Matti
(B) STREET: Maijamaentie 13
(C) CITY: Naantali
(E) COUNTRY: Finland
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(A) NAME: KARP, Matti
(B) STREET: Kampakatu 1
(C) CITY: Kaarina
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-20660

(A) NAME: KURITTU, Jussi
(B) STREET: Puutarhakatu 16 A 20
(C) CITY: Turku
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-20100

(ii) TITLE OF INVENTION: A NEW ASSAY METHOD

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: FI 974235
(B) FILING DATE: 14-NOV-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4846 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Photinus pyralis

(vii) IMMEDIATE SOURCE:
(B) CLONE: pTetLuc1

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: Plasmid

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..3098
(D) OTHER INFORMATION:/standard_name= "Vector pASK75"
/note= "Part of plasmid originating from vector pASK75; feature description below, SEQ ID 9-11."
/citation= ([2])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:3119..4768
(D) OTHER INFORMATION:/product= "Photinus pyralis luciferase"
/citation= ([1])

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Bonin,
(B) TITLE: Photinus pyralis luciferase: vectors that contain a modified luc coding sequence allowing convenient transfer into other systems
(C) JOURNAL: Gene
(D) VOLUME: 141
(F) PAGES: 75-77
(G) DATE: 1994
(K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 3099 TO 4772

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Skerra, A
(B) TITLE: Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli
(C) JOURNAL: Gene
(D) VOLUME: 151
(E) ISSUE: 1-2
(F) PAGES: 131-135
(G) DATE: 30-DEC-1994
(K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 1 TO 3098

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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465 470 475 480

CCC GCC GCC GTT GTT GTT TTG GAG CAC GGA AAG ACG ATG ACG GAA AAA 4606
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 550 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Glu Leu Leu Asn Ser Met Asn Ile Ser Gln Pro Thr Val Val Phe Val
        115                 120                 125

Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro
    130                 135                 140

Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly
145                 150                 155                 160

Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe
                165                 170                 175

Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile
            180                 185                 190

Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val
        195                 200                 205

Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp
    210                 215                 220
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Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val
225 230 235 240

Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu
245 250 255

Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu
260 265 270

Phe Leu Arg Ser Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val
275 280 285

Pro Thr Leu Phe Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr
290 295 300

Asp Leu Ser Asn Leu His Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser
305 310 315 320

Lys Glu Val Gly Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile
325 330 335

Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr
340 345 350

Pro Glu Gly Asp Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe
355 360 365

Phe Glu Ala Lys Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val
370 375 380

Asn Gln Arg Gly Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly
385 390 395 400

Tyr Val Asn Asn Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly
405 410 415

Trp Leu His Ser Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe
420 425 430

Phe Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln
435 440 445

Val Ala Pro Ala Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile
450 455 460

Phe Asp Ala Gly Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu
465 470 475 480

Pro Ala Ala Val Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys
485 490 495

Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu
500 505 510

Arg Gly Gly Val Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly
515 520 525

Lys Leu Asp Ala Arg Lys Ile Arg Glu Ile Leu Ile Lys Ala Lys Lys
530 535 540

Gly Gly Lys Ser Lys Leu
545 550

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10220 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Photorhabdus luminescens

(vii) IMMEDIATE SOURCE:
(B) CLONE: pTetLux1

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:join(1..3190, 10140..10220)
(D) OTHER INFORMATION:/standard_name= "vector pASK75"
/note= "Parts of plasmid originating from vector pASK75; feature description below, SEQ ID NO: 9-11."
/citation= ([2])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:3634..5082
(D) OTHER INFORMATION:/product= "Lux C"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:5097..6017
(D) OTHER INFORMATION:/product= "Lux D"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:6069..7148
(D) OTHER INFORMATION:/product= "Lux A"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:7166..8146
(D) OTHER INFORMATION:/product= "Lux B"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:8256..9437
(D) OTHER INFORMATION:/product= "Lux E"
/citation= ([1])

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Frackman,
(B) TITLE: Cloning, organization and expression of the bioluminescence genes of Xenorhabdus lumiminescenss
(C) JOURNAL: J. Bacteriol.
(D) VOLUME: 172
(F) PAGES: 5767-5773
(G) DATE: 1990
(K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 3191 TO 10139

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Skerra, A
(B) TITLE: Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli
(C) JOURNAL: Gene
(D) VOLUME: 151
(E) ISSUE: 1-2
(F) PAGES: 131-135
(G) DATE: 30-DEC-1994
(K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 1 TO 3190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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GCTTTCTTCC CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG    240
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TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG    360
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CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT   1380
GAGCGTGGCT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC   1440
GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT   1500
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CTAGAGAATG CATTATATGC ACTCAGCGCA GTGGGGCATT TTACTTTAGG TTGCGTATTG 1980
GAAGATCAAG AGCATCAAGT CGCTAAAGAA GAAAGGGAAA CACCTACTAC TGATAGTATG 2040
CCGCCATTAT TACGACAAGC TATCGAATTA TTTGATCACC AAGGTGCAGA GCCAGCCTTC 2100
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CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT 2460
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GCAACTTAAG TTGAAATTAC CCCCATTAAA TGG ATG GCA AAT ATG ACT AAA AAA     3654
                                     Met Ala Asn Met Thr Lys Lys
                                                     555

ATT TCA TTC ATT ATT AAC GGC CAG GTT GAA ATC TTT CCC GAA AGT GAT      3702
Ile Ser Phe Ile Ile Asn Gly Gln Val Glu Ile Phe Pro Glu Ser Asp
        560                 565                 570

GAT TTA GTG CAA TCC ATT AAT TTT GGT GAT AAT AGT GTT TAC CTG CCA      3750
Asp Leu Val Gln Ser Ile Asn Phe Gly Asp Asn Ser Val Tyr Leu Pro
    575                 580                 585

ATA TTG AAT GAC TCT CAT GTA AAA AAC ATT ATT GAT TGT AAT GGA AAT      3798
Ile Leu Asn Asp Ser His Val Lys Asn Ile Ile Asp Cys Asn Gly Asn
590                 595                 600                 605

AAC GAA TTA CGG TTG CAT AAC ATT GTC AAT TTT CTC TAT ACG GTA GGG      3846
Asn Glu Leu Arg Leu His Asn Ile Val Asn Phe Leu Tyr Thr Val Gly
                610                 615                 620

CAA AGA TGG AAA AAT GAA GAA TAC TCA AGA CGC AGG ACA TAC ATT CGT      3894
Gln Arg Trp Lys Asn Glu Glu Tyr Ser Arg Arg Arg Thr Tyr Ile Arg
            625                 630                 635

GAC TTA AAA AAA TAT ATG GGA TAT TCA GAA GAA ATG GCT AAG CTA GAG      3942
Asp Leu Lys Lys Tyr Met Gly Tyr Ser Glu Glu Met Ala Lys Leu Glu
        640                 645                 650

GCC AAT TGG ATA TCT ATG ATT TTA TGT TCT AAA GGC GGC CTT TAT GAT      3990
Ala Asn Trp Ile Ser Met Ile Leu Cys Ser Lys Gly Gly Leu Tyr Asp
    655                 660                 665

GTT GTA GAA AAT GAA CTT GGT TCT CGC CAT ATC ATG GAT GAA TGG CTA      4038
Val Val Glu Asn Glu Leu Gly Ser Arg His Ile Met Asp Glu Trp Leu
670                 675                 680                 685

CCT CAG GAT GAA AGT TAT GTT CGG GCT TTT CCG AAA GGT AAA TCT GTA      4086
Pro Gln Asp Glu Ser Tyr Val Arg Ala Phe Pro Lys Gly Lys Ser Val
                690                 695                 700

CAT CTG TTG GCA GGT AAT GTT CCA TTA TCT GGG ATC ATG TCT ATA TTA      4134
His Leu Leu Ala Gly Asn Val Pro Leu Ser Gly Ile Met Ser Ile Leu
            705                 710                 715

CGC GCA ATT TTA ACT AAG AAT CAG TGT ATT ATA AAA ACA TCG TCA ACC      4182
Arg Ala Ile Leu Thr Lys Asn Gln Cys Ile Ile Lys Thr Ser Ser Thr
        720                 725                 730

GAT CCT TTT ACC GCT AAT GCA TTA GCG TTA AGT TTT ATT GAT GTA GAC      4230
Asp Pro Phe Thr Ala Asn Ala Leu Ala Leu Ser Phe Ile Asp Val Asp
    735                 740                 745

CCT AAT CAT CCG ATA ACG CGC TCT TTA TCT GTT ATA TAT TGG CCC CAC      4278
Pro Asn His Pro Ile Thr Arg Ser Leu Ser Val Ile Tyr Trp Pro His
750                 755                 760                 765

CAA GGT GAT ACA TCA CTC GCA AAA GAA ATT ATG CGA CAT GCG GAT GTT      4326
Gln Gly Asp Thr Ser Leu Ala Lys Glu Ile Met Arg His Ala Asp Val
                770                 775                 780

ATT GTC GCT TGG GGA GGG CCA GAT GCG ATT AAT TGG GCG GTA GAG CAT      4374
Ile Val Ala Trp Gly Gly Pro Asp Ala Ile Asn Trp Ala Val Glu His
            785                 790                 795

GCG CCA TCT TAT GCT GAT GTG ATT AAA TTT GGT TCT AAA AAG AGT CTT      4422
Ala Pro Ser Tyr Ala Asp Val Ile Lys Phe Gly Ser Lys Lys Ser Leu
        800                 805                 810
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TGC ATT ATC GAT AAT CCT GTT GAT TTG ACG TCC GCA GCG ACA GGT GCG      4470
Cys Ile Ile Asp Asn Pro Val Asp Leu Thr Ser Ala Ala Thr Gly Ala
    815                 820                 825

GCT CAT GAT GTT TGT TTT TAC GAT CAG CGA GCT TGT TTT TCT GCC CAA      4518
Ala His Asp Val Cys Phe Tyr Asp Gln Arg Ala Cys Phe Ser Ala Gln
830                 835                 840                 845

AAC ATA TAT TAC ATG GGA AAT CAT TAT GAG GAA TTT AAG TTA GCG TTG      4566
Asn Ile Tyr Tyr Met Gly Asn His Tyr Glu Glu Phe Lys Leu Ala Leu
                850                 855                 860

ATA GAA AAA CTT AAT CTA TAT GCG CAT ATA TTA CCG AAT GCC AAA AAA      4614
Ile Glu Lys Leu Asn Leu Tyr Ala His Ile Leu Pro Asn Ala Lys Lys
            865                 870                 875

GAT TTT GAT GAA AAG GCG GCC TAT TCT TTA GTT CAA AAA GAA AGC TTG      4662
Asp Phe Asp Glu Lys Ala Ala Tyr Ser Leu Val Gln Lys Glu Ser Leu
        880                 885                 890

TTT GCT GGA TTA AAA GTA GAG GTG GAT ATT CAT CAA CGT TGG ATG ATT      4710
Phe Ala Gly Leu Lys Val Glu Val Asp Ile His Gln Arg Trp Met Ile
    895                 900                 905

ATT GAG TCA AAT GCA GGT GTG GAA TTT AAT CAA CCA CTT GGC AGA TGT      4758
Ile Glu Ser Asn Ala Gly Val Glu Phe Asn Gln Pro Leu Gly Arg Cys
910                 915                 920                 925

GTG TAC CTT CAT CAC GTC GAT AAT ATT GAG CAA ATA TTG CCT TAT GTT      4806
Val Tyr Leu His His Val Asp Asn Ile Glu Gln Ile Leu Pro Tyr Val
                930                 935                 940

CAA AAA AAT AAG ACG CAA ACC ATA TCT ATT TTT CCT TGG GAG TCA TCA      4854
Gln Lys Asn Lys Thr Gln Thr Ile Ser Ile Phe Pro Trp Glu Ser Ser
            945                 950                 955

TTT AAA TAT CGA GAT GCG TTA GCA TTA AAA GGT GCG GAA AGG ATT GTA      4902
Phe Lys Tyr Arg Asp Ala Leu Ala Leu Lys Gly Ala Glu Arg Ile Val
        960                 965                 970

GAA GCA GGA ATG AAT AAC ATA TTT CGA GTT GGT GGA TCT CAT GAC GGA      4950
Glu Ala Gly Met Asn Asn Ile Phe Arg Val Gly Gly Ser His Asp Gly
    975                 980                 985

ATG AGA CCG TTG CAA CGA TTA GTG ACA TAT ATT TCT CAT GAA AGG CCA      4998
Met Arg Pro Leu Gln Arg Leu Val Thr Tyr Ile Ser His Glu Arg Pro
990                 995                 1000                1005

TCT AAC TAT ACG GCT AAG GAT GTT GCG GTT GAA ATA GAA CAG ACT CGA      5046
Ser Asn Tyr Thr Ala Lys Asp Val Ala Val Glu Ile Glu Gln Thr Arg
                1010                1015                1020

TTC CTG GAA GAA GAT AAG TTC CTT GTA TTT GTC CCA TAATAGGTAA           5092
Phe Leu Glu Glu Asp Lys Phe Leu Val Phe Val Pro
            1025                1030

AAGT ATG GAA AAT GAA TCA AAA TAT AAA ACC ATC GAC CAC GTT ATT TGT     5141
     Met Glu Asn Glu Ser Lys Tyr Lys Thr Ile Asp His Val Ile Cys
       1               5                  10                  15

GTT GAA GGA AAT AAA AAA ATT CAT GTT TGG GAA ACG CTG CCA GAA GAA      5189
Val Glu Gly Asn Lys Lys Ile His Val Trp Glu Thr Leu Pro Glu Glu
                20                  25                  30

AAC AGC CCA AAG AGA AAG AAT GCC ATT ATT ATT GCG TCT GGT TTT GCC      5237
Asn Ser Pro Lys Arg Lys Asn Ala Ile Ile Ile Ala Ser Gly Phe Ala
            35                  40                  45
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CGC AGG ATG GAT CAT TTT GCT GGT CTG GCG GAA TAT TTA TCG CGG AAT        5285
Arg Arg Met Asp His Phe Ala Gly Leu Ala Glu Tyr Leu Ser Arg Asn
        50                  55                  60

GGA TTT CAT GTG ATC CGC TAT GAT TCG CTT CAC CAC GTT GGA TTG AGT        5333
Gly Phe His Val Ile Arg Tyr Asp Ser Leu His His Val Gly Leu Ser
    65                  70                  75

TCA GGG ACA ATT GAT GAA TTT ACA ATG TCT ATA GGA AAG CAG AGC TTG        5381
Ser Gly Thr Ile Asp Glu Phe Thr Met Ser Ile Gly Lys Gln Ser Leu
80                  85                  90                  95

TTA GCA GTG GTT GAT TGG TTA ACT ACA CGA AAA ATA AAT AAC TTC GGT        5429
Leu Ala Val Val Asp Trp Leu Thr Thr Arg Lys Ile Asn Asn Phe Gly
                100                 105                 110

ATG TTG GCT TCA AGC TTA TCT GCG CGG ATA GCT TAT GCA AGC CTA TCT        5477
Met Leu Ala Ser Ser Leu Ser Ala Arg Ile Ala Tyr Ala Ser Leu Ser
            115                 120                 125

GAA ATC AAT GCT TCG TTT TTA ATC ACC GCA GTC GGT GTT GTT AAC TTA        5525
Glu Ile Asn Ala Ser Phe Leu Ile Thr Ala Val Gly Val Val Asn Leu
        130                 135                 140

AGA TAT TCT CTT GAA AGA GCT TTA GGG TTT GAT TAT CTC AGT CTA CCC        5573
Arg Tyr Ser Leu Glu Arg Ala Leu Gly Phe Asp Tyr Leu Ser Leu Pro
    145                 150                 155

ATT AAT GAA TTG CCG GAT AAT CTA GAT TTT GAA GGC CAT AAA TTG GGT        5621
Ile Asn Glu Leu Pro Asp Asn Leu Asp Phe Glu Gly His Lys Leu Gly
160                 165                 170                 175

GCT GAA GTC TTT GCG AGA GAT TGT CTT GAT TTT GGT TGG GAA GAT TTA        5669
Ala Glu Val Phe Ala Arg Asp Cys Leu Asp Phe Gly Trp Glu Asp Leu
                180                 185                 190

GCT TCT ACA ATT AAT AAC ATG ATG TAT CTT GAT ATA CCG TTT ATT GCT        5717
Ala Ser Thr Ile Asn Asn Met Met Tyr Leu Asp Ile Pro Phe Ile Ala
            195                 200                 205

TTT ACT GCA AAT AAC GAT AAT TGG GTC AAG CAA GAT GAA GTT ATC ACA        5765
Phe Thr Ala Asn Asn Asp Asn Trp Val Lys Gln Asp Glu Val Ile Thr
        210                 215                 220

TTG TTA TCA AAT ATT CGT AGT AAT CGA TGC AAG ATA TAT TCT TTG TTA        5813
Leu Leu Ser Asn Ile Arg Ser Asn Arg Cys Lys Ile Tyr Ser Leu Leu
    225                 230                 235

GGA AGT TCG CAT GAC TTG AGT GAA AAT TTA GTG GTC CTG CGC AAT TTT        5861
Gly Ser Ser His Asp Leu Ser Glu Asn Leu Val Val Leu Arg Asn Phe
240                 245                 250                 255

TAT CAA TCG GTT ACG AAA GCC GCT ATC GCG ATG GAT AAT GAT CAT CTG        5909
Tyr Gln Ser Val Thr Lys Ala Ala Ile Ala Met Asp Asn Asp His Leu
                260                 265                 270

GAT ATT GAT GTT GAT ATT ACT GAA CCG TCA TTT GAA CAT TTA ACT ATT        5957
Asp Ile Asp Val Asp Ile Thr Glu Pro Ser Phe Glu His Leu Thr Ile
            275                 280                 285

GCG ACA GTC AAT GAA CGC CGA ATG AGA ATT GAG ATT GAA AAT CAA GCA        6005
Ala Thr Val Asn Glu Arg Arg Met Arg Ile Glu Ile Glu Asn Gln Ala
        290                 295                 300

ATT TCT CTG TCT TAAAATCTAT TGAGATATTC TATCACTCAA ATAGCAATAT            6057
Ile Ser Leu Ser
    305
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             Met Lys Phe Gly Asn Phe Leu Leu Thr Tyr Gln Pro Pro
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CAA TTT TCT CAA ACA GAG GTA ATG AAA CGT TTG GTT AAA TTA GGT CGC      6155
Gln Phe Ser Gln Thr Glu Val Met Lys Arg Leu Val Lys Leu Gly Arg
    15                  20                  25

ATC TCT GAG GAG TGT GGT TTT GAT ACC GTA TGG TTA CTG GAG CAT CAT      6203
Ile Ser Glu Glu Cys Gly Phe Asp Thr Val Trp Leu Leu Glu His His
30                  35                  40                  45

TTC ACG GAG TTT GGT TTG CTT GGT AAC CCT TAT GTC GCT GCT GCA TAT      6251
Phe Thr Glu Phe Gly Leu Leu Gly Asn Pro Tyr Val Ala Ala Ala Tyr
                50                  55                  60

TTA CTT GGC GCG ACT AAA AAA TTG AAT GTA GGA ACT GCC GCT ATT GTT      6299
Leu Leu Gly Ala Thr Lys Lys Leu Asn Val Gly Thr Ala Ala Ile Val
            65                  70                  75

CTT CCC ACA GCC CAT CCA GTA CGC CAA CTT GAA GAT GTG AAT TTA TTG      6347
Leu Pro Thr Ala His Pro Val Arg Gln Leu Glu Asp Val Asn Leu Leu
        80                  85                  90

GAT CAA ATG TCA AAA GGA CGA TTT CGG TTT GGT ATT TGC CGA GGG CTT      6395
Asp Gln Met Ser Lys Gly Arg Phe Arg Phe Gly Ile Cys Arg Gly Leu
    95                  100                 105

TAC AAC AAG GAC TTT CGC GTA TTC GGC ACA GAT ATG AAT AAC AGT CGC      6443
Tyr Asn Lys Asp Phe Arg Val Phe Gly Thr Asp Met Asn Asn Ser Arg
110                 115                 120                 125

GCC TTA GCG GAA TGC TGG TAC GGG CTG ATA AAG AAT GGC ATG ACA GAG      6491
Ala Leu Ala Glu Cys Trp Tyr Gly Leu Ile Lys Asn Gly Met Thr Glu
                130                 135                 140

GGA TAT ATG GAA GCT GAT AAT GAA CAT ATC AAG TTC CAT AAG GTA AAA      6539
Gly Tyr Met Glu Ala Asp Asn Glu His Ile Lys Phe His Lys Val Lys
            145                 150                 155

GTA AAC CCC GCG GCG TAT AGC AGA GGT GGC GCA CCG GTT TAT GTG GTG      6587
Val Asn Pro Ala Ala Tyr Ser Arg Gly Gly Ala Pro Val Tyr Val Val
        160                 165                 170

GCT GAA TCA GCT TCG ACG ACT GAG TGG GCT GCT CAA TTT GGC CTA CCG      6635
Ala Glu Ser Ala Ser Thr Thr Glu Trp Ala Ala Gln Phe Gly Leu Pro
    175                 180                 185

ATG ATA TTA AGT TGG ATT ATA AAT ACT AAC GAA AAG AAA GCA CAA CTT      6683
Met Ile Leu Ser Trp Ile Ile Asn Thr Asn Glu Lys Lys Ala Gln Leu
190                 195                 200                 205

GAG CTT TAT AAT GAA GTG GCT CAA GAA TAT GGG CAC GAT ATT CAT AAT      6731
Glu Leu Tyr Asn Glu Val Ala Gln Glu Tyr Gly His Asp Ile His Asn
                210                 215                 220

ATC GAC CAT TGC TTA TCA TAT ATA ACA TCT GTA GAT CAT GAC TCA ATT      6779
Ile Asp His Cys Leu Ser Tyr Ile Thr Ser Val Asp His Asp Ser Ile
            225                 230                 235

AAA GCG AAA GAG ATT TGC CGG AAA TTT CTG GGG CAT TGG TAT GAT TCT      6827
Lys Ala Lys Glu Ile Cys Arg Lys Phe Leu Gly His Trp Tyr Asp Ser
        240                 245                 250

TAT GTG AAT GCT ACG ACT ATT TTT GAT GAT TCA GAC CAA ACA AGA GGT      6875
Tyr Val Asn Ala Thr Thr Ile Phe Asp Asp Ser Asp Gln Thr Arg Gly
    255                 260                 265
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TAT GAT TTC AAT AAA GGG CAG TGG CGT GAC TTT GTA TTA AAA GGA CAT      6923
Tyr Asp Phe Asn Lys Gly Gln Trp Arg Asp Phe Val Leu Lys Gly His
270                 275                 280                 285

AAA GAT ACT AAT CGC CGT ATT GAT TAC AGT TAC GAA ATC AAT CCC GTG      6971
Lys Asp Thr Asn Arg Arg Ile Asp Tyr Ser Tyr Glu Ile Asn Pro Val
                290                 295                 300

GGA ACG CCG CAG GAA TGT ATT GAC ATA ATT CAA AAA GAC ATT GAT GCT      7019
Gly Thr Pro Gln Glu Cys Ile Asp Ile Ile Gln Lys Asp Ile Asp Ala
            305                 310                 315

ACA GGA ATA TCA AAT ATT TGT TGT GGA TTT GAA GCT AAT GGA ACA GTA      7067
Thr Gly Ile Ser Asn Ile Cys Cys Gly Phe Glu Ala Asn Gly Thr Val
        320                 325                 330

GAC GAA ATT ATT GCT TCC ATG AAG CTC TTC CAG TCT GAT GTC ATG CCA      7115
Asp Glu Ile Ile Ala Ser Met Lys Leu Phe Gln Ser Asp Val Met Pro
    335                 340                 345

TTT CTT AAA GAA AAA CAA CGT TCG CTA TTA TAT TAGCTAAGGA GAAAGAA       7165
Phe Leu Lys Glu Lys Gln Arg Ser Leu Leu Tyr
350                 355                 360

ATG AAA TTT GGA TTG TTC TTC CTT AAC TTC ATC AAT TCA ACA ACT GTT      7213
Met Lys Phe Gly Leu Phe Phe Leu Asn Phe Ile Asn Ser Thr Thr Val
  1               5                  10                  15

CAA GAA CAA AGT ATA GTT CGC ATG CAG GAA ATA ACG GAG TAT GTT GAT      7261
Gln Glu Gln Ser Ile Val Arg Met Gln Glu Ile Thr Glu Tyr Val Asp
             20                  25                  30

AAG TTG AAT TTT GAA CAG ATT TTA GTG TAT GAA AAT CAT TTT TCA GAT      7309
Lys Leu Asn Phe Glu Gln Ile Leu Val Tyr Glu Asn His Phe Ser Asp
         35                  40                  45

AAT GGT GTT GTC GGC GCT CCT CTG ACT GTT TCT GGT TTT CTG CTC GGT      7357
Asn Gly Val Val Gly Ala Pro Leu Thr Val Ser Gly Phe Leu Leu Gly
     50                  55                  60

TTA ACA GAG AAA ATT AAA ATT GGT TCA TTA AAT CAC ATC ATT ACA ACT      7405
Leu Thr Glu Lys Ile Lys Ile Gly Ser Leu Asn His Ile Ile Thr Thr
 65                  70                  75                  80

CAT CAT CCT GTC GCC ATA GCG GAG GAA GCT TGC TTA TTG GAT CAG TTA      7453
His His Pro Val Ala Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln Leu
                 85                  90                  95

AGT GAA GGG AGA TTT ATT TTA GGG TTT AGT GAT TGC GAA AAA AAA GAT      7501
Ser Glu Gly Arg Phe Ile Leu Gly Phe Ser Asp Cys Glu Lys Lys Asp
            100                 105                 110

GAA ATG CAT TTT TTT AAT CGC CCG GTT GAA TAT CAA CAG CAA CTA TTT      7549
Glu Met His Phe Phe Asn Arg Pro Val Glu Tyr Gln Gln Gln Leu Phe
        115                 120                 125

GAA GAG TGT TAT GAA ATC ATT AAC GAT GCT TTA ACA ACA GGC TAT TGT      7597
Glu Glu Cys Tyr Glu Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr Cys
    130                 135                 140

AAT CCA GAT AAC GAT TTT TAT AGC TTC CCT AAA ATA TCT GTA AAT CCC      7645
Asn Pro Asp Asn Asp Phe Tyr Ser Phe Pro Lys Ile Ser Val Asn Pro
145                 150                 155                 160

CAT GCT TAT ACG CCA GGC GGA CCT CGG AAA TAT GTA ACA GCA ACC AGT      7693
His Ala Tyr Thr Pro Gly Gly Pro Arg Lys Tyr Val Thr Ala Thr Ser
                165                 170                 175
```

CAT CAT ATT GTT GAG TGG GCG GCC AAA AAA GGT ATT CCT CTC ATC TTT 7741
His His Ile Val Glu Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile Phe
180 185 190

AAG TGG GAT GAT TCT AAT GAT GTT AGA TAT GAA TAT GCT GAA AGA TAT 7789
Lys Trp Asp Asp Ser Asn Asp Val Arg Tyr Glu Tyr Ala Glu Arg Tyr
195 200 205

AAA GCC GTT GCG GAT AAA TAT GAC GTT GAC CTA TCA GAG ATA GAC CAT 7837
Lys Ala Val Ala Asp Lys Tyr Asp Val Asp Leu Ser Glu Ile Asp His
210 215 220

CAG TTA ATG ATA TTA GTT AAC TAT AAC GAA GAT AGT AAT AAA GCT AAA 7885
Gln Leu Met Ile Leu Val Asn Tyr Asn Glu Asp Ser Asn Lys Ala Lys
225 230 235 240

CAA GAG ACG CGT GCA TTT ATT AGT GAT TAT GTT CTT GAA ATG CAC CCT 7933
Gln Glu Thr Arg Ala Phe Ile Ser Asp Tyr Val Leu Glu Met His Pro
245 250 255

AAT GAA AAT TTC GAA AAT AAA CTT GAA GAA ATA ATT GCA GAA AAC GCT 7981
Asn Glu Asn Phe Glu Asn Lys Leu Glu Glu Ile Ile Ala Glu Asn Ala
260 265 270

GTC GGA AAT TAT ACG GAG TGT ATA ACT GCG GCT AAG TTG GCA ATT GAA 8029
Val Gly Asn Tyr Thr Glu Cys Ile Thr Ala Ala Lys Leu Ala Ile Glu
275 280 285

AAG TGT GGT GCG AAA AGT GTA TTG CTG TCC TTT GAA CCA ATG AAT GAT 8077
Lys Cys Gly Ala Lys Ser Val Leu Leu Ser Phe Glu Pro Met Asn Asp
290 295 300

TTG ATG AGC CAA AAA AAT GTA ATC AAT ATT GTT GAT GAT AAT ATT AAG 8125
Leu Met Ser Gln Lys Asn Val Ile Asn Ile Val Asp Asp Asn Ile Lys
305 310 315 320

AAG TAC CAC ATG GAA TAT ACC TAATAGATTT CGAGTTGCAG CGAGGCGGCA 8176
Lys Tyr His Met Glu Tyr Thr
325

AGTGAACGAA TCCCCAGGAG CATAGATAAC TATGTGACTG GGGTGAGTGA AAGCAGCCAA 8236

CAAAGCAGCA GCTTGAAAG ATG AAG GGT ATA AAA GAG TAT GAC AGC AGT GCT 8288
Met Lys Gly Ile Lys Glu Tyr Asp Ser Ser Ala
1 5 10

GCC ATA CTT TCT AAT ATT ATC TTG AGG AGT AAA ACA GGT ATG ACT TCA 8336
Ala Ile Leu Ser Asn Ile Ile Leu Arg Ser Lys Thr Gly Met Thr Ser
15 20 25

TAT GTT GAT AAA CAA GAA ATT ACA GCA AGC TCA GAA ATT GAT GAT TTG 8384
Tyr Val Asp Lys Gln Glu Ile Thr Ala Ser Ser Glu Ile Asp Asp Leu
30 35 40

ATT TTT TCG AGC GAT CCA TTA GTG TGG TCT TAC GAC GAG CAG GAA AAA 8432
Ile Phe Ser Ser Asp Pro Leu Val Trp Ser Tyr Asp Glu Gln Glu Lys
45 50 55

ATC AGA AAG AAA CTT GTG CTT GAT GCA TTT CGT AAT CAT TAT AAA CAT 8480
Ile Arg Lys Lys Leu Val Leu Asp Ala Phe Arg Asn His Tyr Lys His
60 65 70 75

TGT CGA GAA TAT CGT CAC TAC TGT CAG GCA CAC AAA GTA GAT GAC AAT 8528
Cys Arg Glu Tyr Arg His Tyr Cys Gln Ala His Lys Val Asp Asp Asn
80 85 90

ATT ACG GAA ATT GAT GAC ATA CCT GTA TTC CCA ACA TCG GTT TTT AAG 8576
Ile Thr Glu Ile Asp Asp Ile Pro Val Phe Pro Thr Ser Val Phe Lys
95 100 105

TTT ACT CGC TTA TTA ACT TCT CAG GAA AAC GAG ATT GAA AGT TGG TTT 8624
Phe Thr Arg Leu Leu Thr Ser Gln Glu Asn Glu Ile Glu Ser Trp Phe
110 115 120

ACC AGT AGC GGC ACG AAT GGT TTA AAA AGT CAG GTG GCG CGT GAC AGA 8672
Thr Ser Ser Gly Thr Asn Gly Leu Lys Ser Gln Val Ala Arg Asp Arg
125 130 135

TTA AGT ATT GAG AGA CTC TTA GGC TCT GTG AGT TAT GGC ATG AAA TAT 8720
Leu Ser Ile Glu Arg Leu Leu Gly Ser Val Ser Tyr Gly Met Lys Tyr
140 145 150 155

GTT GGT AGT TGG TTT GAT CAT CAA ATA GAA TTA GTC AAT TTG GGA CCA 8768
Val Gly Ser Trp Phe Asp His Gln Ile Glu Leu Val Asn Leu Gly Pro
160 165 170

GAT AGA TTT AAT GCT CAT AAT ATT TGG TTT AAA TAT GTT ATG AGT TTG 8816
Asp Arg Phe Asn Ala His Asn Ile Trp Phe Lys Tyr Val Met Ser Leu
175 180 185

GTG GAA TTG TTA TAT CCT ACG ACA TTT ACC GTA ACA GAA GAA CGA ATA 8864
Val Glu Leu Leu Tyr Pro Thr Thr Phe Thr Val Thr Glu Glu Arg Ile
190 195 200

GAT TTT GTT AAA ACA TTG AAT AGT CTT GAA CGA ATA AAA AAT CAA GGG 8912
Asp Phe Val Lys Thr Leu Asn Ser Leu Glu Arg Ile Lys Asn Gln Gly
205 210 215

AAA GAT CTT TGT CTT ATT GGT TCG CCA TAC TTT ATT TAT TTA CTC TGC 8960
Lys Asp Leu Cys Leu Ile Gly Ser Pro Tyr Phe Ile Tyr Leu Leu Cys
220 225 230 235

CAT TAT ATG AAA GAT AAA AAA ATC TCA TTT TCT GGA GAT AAA AGC CTT 9008
His Tyr Met Lys Asp Lys Lys Ile Ser Phe Ser Gly Asp Lys Ser Leu
240 245 250

TAT ATC ATA ACC GGA GGC GGC TGG AAA AGT TAC GAA AAA GAA TCT CTG 9056
Tyr Ile Ile Thr Gly Gly Gly Trp Lys Ser Tyr Glu Lys Glu Ser Leu
255 260 265

AAA CGT GAT GAT TTC AAT CAT CTT TTA TTT GAT ACT TTC AAT CTC AGT 9104
Lys Arg Asp Asp Phe Asn His Leu Leu Phe Asp Thr Phe Asn Leu Ser
270 275 280

GAT ATT AGT CAG ATC CGA GAT ATA TTT AAT CAA GTT GAA CTC AAC ACT 9152
Asp Ile Ser Gln Ile Arg Asp Ile Phe Asn Gln Val Glu Leu Asn Thr
285 290 295

TGT TTC TTT GAG GAT GAA ATG CAG CGT AAA CAT GTT CCG CCG TGG GTA 9200
Cys Phe Phe Glu Asp Glu Met Gln Arg Lys His Val Pro Pro Trp Val
300 305 310 315

TAT GCG CGA GCG CTT GAT CCT GAA ACG TTG AAA CCT GTA CCT GAT GGA 9248
Tyr Ala Arg Ala Leu Asp Pro Glu Thr Leu Lys Pro Val Pro Asp Gly
320 325 330

ACG CCG GGG TTG ATG AGT TAT ATG GAT GCG TCA GCA ACC AGT TAT CCA 9296
Thr Pro Gly Leu Met Ser Tyr Met Asp Ala Ser Ala Thr Ser Tyr Pro
335 340 345

GCA TTT ATT GTT ACC GAT GAT GTC GGG ATA ATT AGC AGA GAA TAT GGT 9344
Ala Phe Ile Val Thr Asp Asp Val Gly Ile Ile Ser Arg Glu Tyr Gly
350 355 360

AAG TAT CCC GGC GTG CTC GTT GAA ATT TTA CGT CGC GTC AAT ACG AGG 9392
Lys Tyr Pro Gly Val Leu Val Glu Ile Leu Arg Arg Val Asn Thr Arg
365 370 375

ACG CAG AAA GGG TGT GCT TTA AGC TTA ACC GAA GCG TTT GAT AGT 9437
Thr Gln Lys Gly Cys Ala Leu Ser Leu Thr Glu Ala Phe Asp Ser
380 385 390

TGATATCCTT TGCCTAATTG TAAGTGGAAT GCTTGCGTTA TATAAATCTG AATGACATCT 9497

ACACTTTACA AAATTCTCCA AAACATCCAC ATTTGGGTAC TTGATAGAGG TTTATGGGGT 9557

TGGCTTAACA TTGTTCTCAT TGTTATTATT GGCTCAAAGC AAAAGGAGAT AACATGAAAA 9617

AATTGGCAGT TATGCTTGCA TTGGGAATGA TTAGCTTTGG TGCAATGGCA GTTGATGGGT 9677

ATAAAGATGC AAAGTTTGGC ATGACAGAAG AAGAGTTTCT TTCGAAGAGG TTATGTGATT 9737

TTGAAAAATT TGAGGGAGAT TCTCGAATAG AAGAAGTATC ACTTTATTCA TGTTCTGACT 9797

TTTCGTTTGC TAACAAAAAG CGTGAAGCAA TGGCATTTTT TTTAAATGGG AAATTTAAAA 9857

GATTAGAGAT TAATATTGGC AGACTTGTGA AGCCAGTAAG CAAATCGTTA ACGAAAAAGT 9917

ACGGAGATGG ATCATCGTAT CCATCAAAAG AAGAATTTGA GAACGCGCTA AAATACAATG 9977

GAACTATGTC TATAGGTTAT GATAATAATA CGGTATTAGT TGATATACAT ATAATATGTG 10037

GCAAAGAAGG CATAGAAACC AGTCAACTGA TTTATACGAG TCCAGATGTT TATACGCTCC 10097

CAGATTTCGG AGAAAAAATC CAGGAATTAA AGGGATTAAA GGAATTCGAG CTCGGTACCC 10157

GGGGATCCCT CGAGGTCGAC CTGCAGGCAG CGCTTGGCGT CACCCGCAGT TCGGTGGTTA 10217

ATA 10220

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala Asn Met Thr Lys Lys Ile Ser Phe Ile Ile Asn Gly Gln Val
1 5 10 15

Glu Ile Phe Pro Glu Ser Asp Asp Leu Val Gln Ser Ile Asn Phe Gly
20 25 30

Asp Asn Ser Val Tyr Leu Pro Ile Leu Asn Asp Ser His Val Lys Asn
35 40 45

Ile Ile Asp Cys Asn Gly Asn Asn Glu Leu Arg Leu His Asn Ile Val
50 55 60

Asn Phe Leu Tyr Thr Val Gly Gln Arg Trp Lys Asn Glu Glu Tyr Ser
65 70 75 80

Arg Arg Arg Thr Tyr Ile Arg Asp Leu Lys Lys Tyr Met Gly Tyr Ser
85 90 95

Glu Glu Met Ala Lys Leu Glu Ala Asn Trp Ile Ser Met Ile Leu Cys
100 105 110

```
Ser Lys Gly Gly Leu Tyr Asp Val Val Glu Asn Glu Leu Gly Ser Arg
        115                 120                 125

His Ile Met Asp Glu Trp Leu Pro Gln Asp Glu Ser Tyr Val Arg Ala
    130                 135                 140

Phe Pro Lys Gly Lys Ser Val His Leu Leu Ala Gly Asn Val Pro Leu
145                 150                 155                 160

Ser Gly Ile Met Ser Ile Leu Arg Ala Ile Leu Thr Lys Asn Gln Cys
                165                 170                 175

Ile Ile Lys Thr Ser Ser Thr Asp Pro Phe Thr Ala Asn Ala Leu Ala
            180                 185                 190

Leu Ser Phe Ile Asp Val Asp Pro Asn His Pro Ile Thr Arg Ser Leu
        195                 200                 205

Ser Val Ile Tyr Trp Pro His Gln Gly Asp Thr Ser Leu Ala Lys Glu
    210                 215                 220

Ile Met Arg His Ala Asp Val Ile Val Ala Trp Gly Gly Pro Asp Ala
225                 230                 235                 240

Ile Asn Trp Ala Val Glu His Ala Pro Ser Tyr Ala Asp Val Ile Lys
                245                 250                 255

Phe Gly Ser Lys Lys Ser Leu Cys Ile Ile Asp Asn Pro Val Asp Leu
            260                 265                 270

Thr Ser Ala Ala Thr Gly Ala Ala His Asp Val Cys Phe Tyr Asp Gln
        275                 280                 285

Arg Ala Cys Phe Ser Ala Gln Asn Ile Tyr Tyr Met Gly Asn His Tyr
    290                 295                 300

Glu Glu Phe Lys Leu Ala Leu Ile Glu Lys Leu Asn Leu Tyr Ala His
305                 310                 315                 320

Ile Leu Pro Asn Ala Lys Lys Asp Phe Asp Glu Lys Ala Ala Tyr Ser
                325                 330                 335

Leu Val Gln Lys Glu Ser Leu Phe Ala Gly Leu Lys Val Glu Val Asp
            340                 345                 350

Ile His Gln Arg Trp Met Ile Ile Glu Ser Asn Ala Gly Val Glu Phe
        355                 360                 365

Asn Gln Pro Leu Gly Arg Cys Val Tyr Leu His His Val Asp Asn Ile
    370                 375                 380

Glu Gln Ile Leu Pro Tyr Val Gln Lys Asn Lys Thr Gln Thr Ile Ser
385                 390                 395                 400

Ile Phe Pro Trp Glu Ser Ser Phe Lys Tyr Arg Asp Ala Leu Ala Leu
                405                 410                 415

Lys Gly Ala Glu Arg Ile Val Glu Ala Gly Met Asn Asn Ile Phe Arg
            420                 425                 430

Val Gly Gly Ser His Asp Gly Met Arg Pro Leu Gln Arg Leu Val Thr
        435                 440                 445

Tyr Ile Ser His Glu Arg Pro Ser Asn Tyr Thr Ala Lys Asp Val Ala
    450                 455                 460

Val Glu Ile Glu Gln Thr Arg Phe Leu Glu Glu Asp Lys Phe Leu Val
465                 470                 475                 480
```

Phe Val Pro

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 307 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Glu Asn Glu Ser Lys Tyr Lys Thr Ile Asp His Val Ile Cys Val
  1               5                  10                  15

Glu Gly Asn Lys Lys Ile His Val Trp Glu Thr Leu Pro Glu Glu Asn
             20                  25                  30

Ser Pro Lys Arg Lys Asn Ala Ile Ile Ile Ala Ser Gly Phe Ala Arg
         35                  40                  45

Arg Met Asp His Phe Ala Gly Leu Ala Glu Tyr Leu Ser Arg Asn Gly
     50                  55                  60

Phe His Val Ile Arg Tyr Asp Ser Leu His His Val Gly Leu Ser Ser
 65                  70                  75                  80

Gly Thr Ile Asp Glu Phe Thr Met Ser Ile Gly Lys Gln Ser Leu Leu
                 85                  90                  95

Ala Val Val Asp Trp Leu Thr Thr Arg Lys Ile Asn Asn Phe Gly Met
            100                 105                 110

Leu Ala Ser Ser Leu Ser Ala Arg Ile Ala Tyr Ala Ser Leu Ser Glu
        115                 120                 125

Ile Asn Ala Ser Phe Leu Ile Thr Ala Val Gly Val Val Asn Leu Arg
    130                 135                 140

Tyr Ser Leu Glu Arg Ala Leu Gly Phe Asp Tyr Leu Ser Leu Pro Ile
145                 150                 155                 160

Asn Glu Leu Pro Asp Asn Leu Asp Phe Glu Gly His Lys Leu Gly Ala
                165                 170                 175

Glu Val Phe Ala Arg Asp Cys Leu Asp Phe Gly Trp Glu Asp Leu Ala
            180                 185                 190

Ser Thr Ile Asn Asn Met Met Tyr Leu Asp Ile Pro Phe Ile Ala Phe
        195                 200                 205

Thr Ala Asn Asn Asp Asn Trp Val Lys Gln Asp Glu Val Ile Thr Leu
    210                 215                 220

Leu Ser Asn Ile Arg Ser Asn Arg Cys Lys Ile Tyr Ser Leu Leu Gly
225                 230                 235                 240

Ser Ser His Asp Leu Ser Glu Asn Leu Val Val Leu Arg Asn Phe Tyr
                245                 250                 255

Gln Ser Val Thr Lys Ala Ala Ile Ala Met Asp Asn Asp His Leu Asp
            260                 265                 270

Ile Asp Val Asp Ile Thr Glu Pro Ser Phe Glu His Leu Thr Ile Ala
        275                 280                 285
```

```
Thr Val Asn Glu Arg Arg Met Arg Ile Glu Ile Glu Asn Gln Ala Ile
    290                 295                     300

Ser Leu Ser
305
```

(2) INFORMATION FOR SEQ ID NO: 6:

```
        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 360 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
```

```
Met Lys Phe Gly Asn Phe Leu Leu Thr Tyr Gln Pro Pro Gln Phe Ser
  1               5                  10                  15

Gln Thr Glu Val Met Lys Arg Leu Val Lys Leu Gly Arg Ile Ser Glu
             20                  25                  30

Glu Cys Gly Phe Asp Thr Val Trp Leu Leu Glu His His Phe Thr Glu
         35                  40                  45

Phe Gly Leu Leu Gly Asn Pro Tyr Val Ala Ala Ala Tyr Leu Leu Gly
     50                  55                  60

Ala Thr Lys Lys Leu Asn Val Gly Thr Ala Ala Ile Val Leu Pro Thr
 65                  70                  75                      80

Ala His Pro Val Arg Gln Leu Glu Asp Val Asn Leu Leu Asp Gln Met
                 85                  90                  95

Ser Lys Gly Arg Phe Arg Phe Gly Ile Cys Arg Gly Leu Tyr Asn Lys
            100                 105                 110

Asp Phe Arg Val Phe Gly Thr Asp Met Asn Asn Ser Arg Ala Leu Ala
        115                 120                 125

Glu Cys Trp Tyr Gly Leu Ile Lys Asn Gly Met Thr Glu Gly Tyr Met
    130                 135                 140

Glu Ala Asp Asn Glu His Ile Lys Phe His Lys Val Lys Val Asn Pro
145                 150                 155                     160

Ala Ala Tyr Ser Arg Gly Gly Ala Pro Val Tyr Val Val Ala Glu Ser
                165                 170                 175

Ala Ser Thr Thr Glu Trp Ala Ala Gln Phe Gly Leu Pro Met Ile Leu
            180                 185                 190

Ser Trp Ile Ile Asn Thr Asn Glu Lys Lys Ala Gln Leu Glu Leu Tyr
        195                 200                 205

Asn Glu Val Ala Gln Glu Tyr Gly His Asp Ile His Asn Ile Asp His
    210                 215                 220

Cys Leu Ser Tyr Ile Thr Ser Val Asp His Asp Ser Ile Lys Ala Lys
225                 230                 235                     240

Glu Ile Cys Arg Lys Phe Leu Gly His Trp Tyr Asp Ser Tyr Val Asn
                245                 250                 255

Ala Thr Thr Ile Phe Asp Asp Ser Asp Gln Thr Arg Gly Tyr Asp Phe
            260                 265                 270
```

```
Asn Lys Gly Gln Trp Arg Asp Phe Val Leu Lys Gly His Lys Asp Thr
        275                 280                 285

Asn Arg Arg Ile Asp Tyr Ser Tyr Glu Ile Asn Pro Val Gly Thr Pro
    290                 295                 300

Gln Glu Cys Ile Asp Ile Ile Gln Lys Asp Ile Asp Ala Thr Gly Ile
305                 310                 315                 320

Ser Asn Ile Cys Cys Gly Phe Glu Ala Asn Gly Thr Val Asp Glu Ile
                325                 330                 335

Ile Ala Ser Met Lys Leu Phe Gln Ser Asp Val Met Pro Phe Leu Lys
            340                 345                 350

Glu Lys Gln Arg Ser Leu Leu Tyr
        355                 360
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Lys Phe Gly Leu Phe Phe Leu Asn Phe Ile Asn Ser Thr Thr Val
  1               5                  10                  15

Gln Glu Gln Ser Ile Val Arg Met Gln Glu Ile Thr Glu Tyr Val Asp
            20                  25                  30

Lys Leu Asn Phe Glu Gln Ile Leu Val Tyr Glu Asn His Phe Ser Asp
        35                  40                  45

Asn Gly Val Val Gly Ala Pro Leu Thr Val Ser Gly Phe Leu Leu Gly
    50                  55                  60

Leu Thr Glu Lys Ile Lys Ile Gly Ser Leu Asn His Ile Ile Thr Thr
65                  70                  75                  80

His His Pro Val Ala Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln Leu
                85                  90                  95

Ser Glu Gly Arg Phe Ile Leu Gly Phe Ser Asp Cys Glu Lys Lys Asp
            100                 105                 110

Glu Met His Phe Phe Asn Arg Pro Val Glu Tyr Gln Gln Gln Leu Phe
        115                 120                 125

Glu Glu Cys Tyr Glu Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr Cys
    130                 135                 140

Asn Pro Asp Asn Asp Phe Tyr Ser Phe Pro Lys Ile Ser Val Asn Pro
145                 150                 155                 160

His Ala Tyr Thr Pro Gly Gly Pro Arg Lys Tyr Val Thr Ala Thr Ser
                165                 170                 175

His His Ile Val Glu Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile Phe
            180                 185                 190

Lys Trp Asp Asp Ser Asn Asp Val Arg Tyr Glu Tyr Ala Glu Arg Tyr
        195                 200                 205
```

```
Lys Ala Val Ala Asp Lys Tyr Asp Val Asp Leu Ser Glu Ile Asp His
    210                 215                 220

Gln Leu Met Ile Leu Val Asn Tyr Asn Glu Asp Ser Asn Lys Ala Lys
225                 230                 235                 240

Gln Glu Thr Arg Ala Phe Ile Ser Asp Tyr Val Leu Glu Met His Pro
                245                 250                 255

Asn Glu Asn Phe Glu Asn Lys Leu Glu Glu Ile Ile Ala Glu Asn Ala
            260                 265                 270

Val Gly Asn Tyr Thr Glu Cys Ile Thr Ala Ala Lys Leu Ala Ile Glu
        275                 280                 285

Lys Cys Gly Ala Lys Ser Val Leu Leu Ser Phe Glu Pro Met Asn Asp
    290                 295                 300

Leu Met Ser Gln Lys Asn Val Ile Asn Ile Val Asp Asp Asn Ile Lys
305                 310                 315                 320

Lys Tyr His Met Glu Tyr Thr
                325
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 394 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Lys Gly Ile Lys Glu Tyr Asp Ser Ser Ala Ala Ile Leu Ser Asn
  1               5                  10                  15

Ile Ile Leu Arg Ser Lys Thr Gly Met Thr Ser Tyr Val Asp Lys Gln
             20                  25                  30

Glu Ile Thr Ala Ser Ser Glu Ile Asp Asp Leu Ile Phe Ser Ser Asp
         35                  40                  45

Pro Leu Val Trp Ser Tyr Asp Glu Gln Glu Lys Ile Arg Lys Lys Leu
     50                  55                  60

Val Leu Asp Ala Phe Arg Asn His Tyr Lys His Cys Arg Glu Tyr Arg
 65                  70                  75                  80

His Tyr Cys Gln Ala His Lys Val Asp Asp Asn Ile Thr Glu Ile Asp
                 85                  90                  95

Asp Ile Pro Val Phe Pro Thr Ser Val Phe Lys Phe Thr Arg Leu Leu
            100                 105                 110

Thr Ser Gln Glu Asn Glu Ile Glu Ser Trp Phe Thr Ser Ser Gly Thr
        115                 120                 125

Asn Gly Leu Lys Ser Gln Val Ala Arg Asp Arg Leu Ser Ile Glu Arg
    130                 135                 140

Leu Leu Gly Ser Val Ser Tyr Gly Met Lys Tyr Val Gly Ser Trp Phe
145                 150                 155                 160

Asp His Gln Ile Glu Leu Val Asn Leu Gly Pro Asp Arg Phe Asn Ala
                165                 170                 175
```

```
His Asn Ile Trp Phe Lys Tyr Val Met Ser Leu Val Glu Leu Leu Tyr
            180                 185                 190

Pro Thr Thr Phe Thr Val Thr Glu Glu Arg Ile Asp Phe Val Lys Thr
        195                 200                 205

Leu Asn Ser Leu Glu Arg Ile Lys Asn Gln Gly Lys Asp Leu Cys Leu
    210                 215                 220

Ile Gly Ser Pro Tyr Phe Ile Tyr Leu Leu Cys His Tyr Met Lys Asp
225                 230                 235                 240

Lys Lys Ile Ser Phe Ser Gly Asp Lys Ser Leu Tyr Ile Ile Thr Gly
                245                 250                 255

Gly Gly Trp Lys Ser Tyr Glu Lys Glu Ser Leu Lys Arg Asp Asp Phe
            260                 265                 270

Asn His Leu Leu Phe Asp Thr Phe Asn Leu Ser Asp Ile Ser Gln Ile
        275                 280                 285

Arg Asp Ile Phe Asn Gln Val Glu Leu Asn Thr Cys Phe Phe Glu Asp
    290                 295                 300

Glu Met Gln Arg Lys His Val Pro Pro Trp Val Tyr Ala Arg Ala Leu
305                 310                 315                 320

Asp Pro Glu Thr Leu Lys Pro Val Pro Asp Gly Thr Pro Gly Leu Met
                325                 330                 335

Ser Tyr Met Asp Ala Ser Ala Thr Ser Tyr Pro Ala Phe Ile Val Thr
            340                 345                 350

Asp Asp Val Gly Ile Ile Ser Arg Glu Tyr Gly Lys Tyr Pro Gly Val
        355                 360                 365

Leu Val Glu Ile Leu Arg Arg Val Asn Thr Arg Thr Gln Lys Gly Cys
    370                 375                 380

Ala Leu Ser Leu Thr Glu Ala Phe Asp Ser
385                 390
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3098 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
- (B) CLONE: pASK75

(viii) POSITION IN GENOME:
- (A) CHROMOSOME/SEGMENT: vector

(ix) FEATURE:
- (A) NAME/KEY: promoter
- (B) LOCATION:542..672
- (D) OTHER INFORMATION:/function= "beta-la promoter"
  /label= beta-la
  /citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:673..1530
(D) OTHER INFORMATION:/product= "beta-la"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1543..2163
(D) OTHER INFORMATION:/product= "tetR"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:2713..2950
(D) OTHER INFORMATION:/function= "ORI"
/label= ORI
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION:2976..3073
(D) OTHER INFORMATION:/function= "p tetA promoter"
/citation= ([1])

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Skerra, A
(B) TITLE: Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli
(C) JOURNAL: Gene
(D) VOLUME: 151
(E) ISSUE: 1-2
(F) PAGES: 131-135
(G) DATE: 30-DEC-1994
(K) RELEVANT RESIDUES IN SEQ ID NO: 9: FROM 1 TO 3098

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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AGCTTGACCT GTGAAGTGAA AAATGGCGCA CATTGTGCGA CATTTTTTTT GTCTGCCGTT      60

TACCGCTACT GCGTCACGGA TCTCCACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT     120

GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC     180

GCTTTCTTCC CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG     240

GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT     300

TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG     360

TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT     420

ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA     480

AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT     540

TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA     600

CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA     660

AAAAGGAAGA GT ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC        708
              Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro
              395                 400                 405
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TTT TTT GCG GCA TTT TGC CTT CCT GTT TTT GCT CAC CCA GAA ACG CTG      756
Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu
            410                 415                 420

GTG AAA GTA AAA GAT GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC      804
Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr
        425                 430                 435

ATC GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT GAG AGT TTT CGC CCC      852
Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro
    440                 445                 450

GAA GAA CGT TTT CCA ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC      900
Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly
455                 460                 465                 470

GCG GTA TTA TCC CGT ATT GAC GCC GGG CAA GAG CAA CTC GGT CGC CGC      948
Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg
                475                 480                 485

ATA CAC TAT TCT CAG AAT GAC TTG GTT GAG TAC TCA CCA GTC ACA GAA      996
Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu
            490                 495                 500

AAG CAT CTT ACG GAT GGC ATG ACA GTA AGA GAA TTA TGC AGT GCT GCC     1044
Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala
        505                 510                 515

ATA ACC ATG AGT GAT AAC ACT GCG GCC AAC TTA CTT CTG ACA ACG ATC     1092
Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile
    520                 525                 530

GGA GGA CCG AAG GAG CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT CAT     1140
Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His
535                 540                 545                 550

GTA ACT CGC CTT GAT CGT TGG GAA CCG GAG CTG AAT GAA GCC ATA CCA     1188
Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro
                555                 560                 565

AAC GAC GAG CGT GAC ACC ACG ATG CCT GTA GCA ATG GCA ACA ACG TTG     1236
Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu
            570                 575                 580

CGC AAA CTA TTA ACT GGC GAA CTA CTT ACT CTA GCT TCC CGG CAA CAA     1284
Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln
        585                 590                 595

TTG ATA GAC TGG ATG GAG GCG GAT AAA GTT GCA GGA CCA CTT CTG CGC     1332
Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg
    600                 605                 610

TCG GCC CTT CCG GCT GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC GGT     1380
Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly
615                 620                 625                 630

GAG CGT GGC TCT CGC GGT ATC ATT GCA GCA CTG GGG CCA GAT GGT AAG     1428
Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys
                635                 640                 645

CCC TCC CGT ATC GTA GTT ATC TAC ACG ACG GGG AGT CAG GCA ACT ATG     1476
Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met
            650                 655                 660

GAT GAA CGA AAT AGA CAG ATC GCT GAG ATA GGT GCC TCA CTG ATT AAG     1524
Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys
        665                 670                 675
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CAT TGG TAGGAATTAA TG ATG TCT CGT TTA GAT AAA AGT AAA GTG ATT      1572
His Trp               Met Ser Arg Leu Asp Lys Ser Lys Val Ile
    680                 1               5                10

AAC AGC GCA TTA GAG CTG CTT AAT GAG GTC GGA ATC GAA GGT TTA ACA    1620
Asn Ser Ala Leu Glu Leu Leu Asn Glu Val Gly Ile Glu Gly Leu Thr
                15                  20                  25

ACC CGT AAA CTC GCC CAG AAG CTA GGT GTA GAG CAG CCT ACA TTG TAT    1668
Thr Arg Lys Leu Ala Gln Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr
            30                  35                  40

TGG CAT GTA AAA AAT AAG CGG GCT TTG CTC GAC GCC TTA GCC ATT GAG    1716
Trp His Val Lys Asn Lys Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu
        45                  50                  55

ATG TTA GAT AGG CAC CAT ACT CAC TTT TGC CCT TTA GAA GGG GAA AGC    1764
Met Leu Asp Arg His His Thr His Phe Cys Pro Leu Glu Gly Glu Ser
    60                  65                  70

TGG CAA GAT TTT TTA CGT AAT AAC GCT AAA AGT TTT AGA TGT GCT TTA    1812
Trp Gln Asp Phe Leu Arg Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu
75                  80                  85                  90

CTA AGT CAT CGC GAT GGA GCA AAA GTA CAT TTA GGT ACA CGG CCT ACA    1860
Leu Ser His Arg Asp Gly Ala Lys Val His Leu Gly Thr Arg Pro Thr
                95                  100                 105

GAA AAA CAG TAT GAA ACT CTC GAA AAT CAA TTA GCC TTT TTA TGC CAA    1908
Glu Lys Gln Tyr Glu Thr Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln
            110                 115                 120

CAA GGT TTT TCA CTA GAG AAT GCA TTA TAT GCA CTC AGC GCA GTG GGG    1956
Gln Gly Phe Ser Leu Glu Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly
        125                 130                 135

CAT TTT ACT TTA GGT TGC GTA TTG GAA GAT CAA GAG CAT CAA GTC GCT    2004
His Phe Thr Leu Gly Cys Val Leu Glu Asp Gln Glu His Gln Val Ala
    140                 145                 150

AAA GAA GAA AGG GAA ACA CCT ACT ACT GAT AGT ATG CCG CCA TTA TTA    2052
Lys Glu Glu Arg Glu Thr Pro Thr Thr Asp Ser Met Pro Pro Leu Leu
155                 160                 165                 170

CGA CAA GCT ATC GAA TTA TTT GAT CAC CAA GGT GCA GAG CCA GCC TTC    2100
Arg Gln Ala Ile Glu Leu Phe Asp His Gln Gly Ala Glu Pro Ala Phe
                175                 180                 185

TTA TTC GGC CTT GAA TTG ATC ATA TGC GGA TTA GAA AAA CAA CTT AAA    2148
Leu Phe Gly Leu Glu Leu Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys
            190                 195                 200

TGT GAA AGT GGG TCT TAAAAGCAGC ATAACCTTTT TCCGTGATGG TAACTTCACT    2203
Cys Glu Ser Gly Ser
        205

AGTTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC  2263

GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  2323

ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  2383

TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAACT GGCTTCAGCA  2443

GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  2503

ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  2563
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GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC 2623

AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA 2683

CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA 2743

AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC 2803

CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC 2863

GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG 2923

CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA CATGACCCGA CACCATCGAA 2983

TGGCCAGATG ATTAATTCCT AATTTTTGTT GACACTCTAT CATTGATAGA GTTATTTTAC 3043

CACTCCCTAT CAGTGATAGA GAAAAGTGAA ATGAATAGTT CGACAAAAAT CTAGA 3098

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 286 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
1 5 10 15

Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
20 25 30

Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
35 40 45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
50 55 60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
65 70 75 80

Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
85 90 95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
100 105 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
115 120 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
130 135 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145 150 155 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
165 170 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
180 185 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
195 200 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
210 215 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
275 280 285

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu
1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln
20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys
35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His
50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg
65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly
85 90 95

Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr
100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu
115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys
130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr
145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu
165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu
180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser
195 200 205

CLAIMS

1. A method for the determination of a tetracycline in a sample characterized in that

- the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
- detecting the luminescense emitted from the intact cells, and
- comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
- wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.

2. The method according to claim 1 characterized in that the cells are *Escherichia coli*.

3. The method according to claim 1 or 2 characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

4. The method according to claim 3 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO: 3).

5. The method according to claim 1 or 2 characterized in that

- the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10, and that

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- D-luciferin is added to the mixture of the sample and the cells in order to initiate the luminescence of the cells.

6. The method according to claim 5 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO: 1).

7. The method according to any of the claims 1 - 6 characterized in that the sensitivity of the analysis with respect to the tetracycline is controlled by
- increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, or
- adjusting the pH, or
- combined adjusting of the divalent metal ion concentration and the pH.

8. The method according to any of the claims 1 - 6 characterized in that the sensitivity of the analysis with respect to the tetracycline derivative is increased by the use of cells which are especially antibiotic sensitive mutant strains.

9. The method according to any of the claims 1 - 8 characterized in that the luminescence is measured using an X-ray or polaroid film, a CCD-camera, a liquid scintillation counter or a luminometer.

10. The method according to any of the claims 1 - 9 characterized in that the sample to be analyzed is milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the like.

11. A recombinant prokaryotic cell characterized in that it encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme,

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tetracycline repressor and tetracycline promoter, and that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

12. The cell according to claim 11 characterized in that it is *Escherichia coli.*

13. The cell according to claim 11 or 12, characterized in that it is in dried form, e.g. in lyophilized form.

14. A plasmid characterized in that it comprises the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

15. A plasmid according to claim 14 characterized in that it is pTetLux1 (SEQ ID NO: 3).

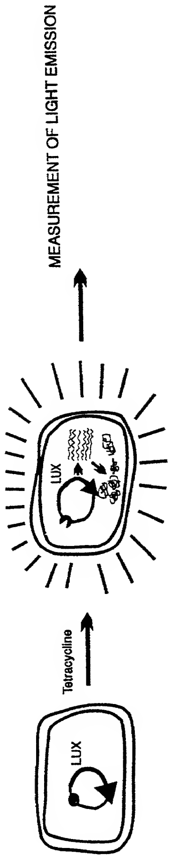
LUX
Tetracycline
LUX
MEASUREMENT OF LIGHT EMISSION

FIG. 1a

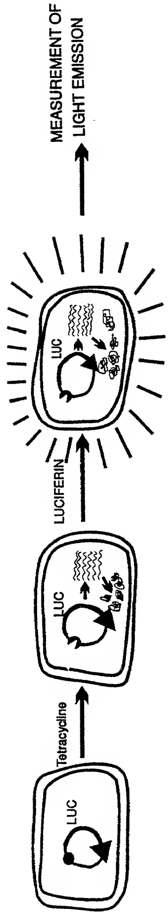
LUC
Tetracycline
LUC
LUCIFERIN
LUC
MEASUREMENT OF
LIGHT EMISSION

FIG. 1b

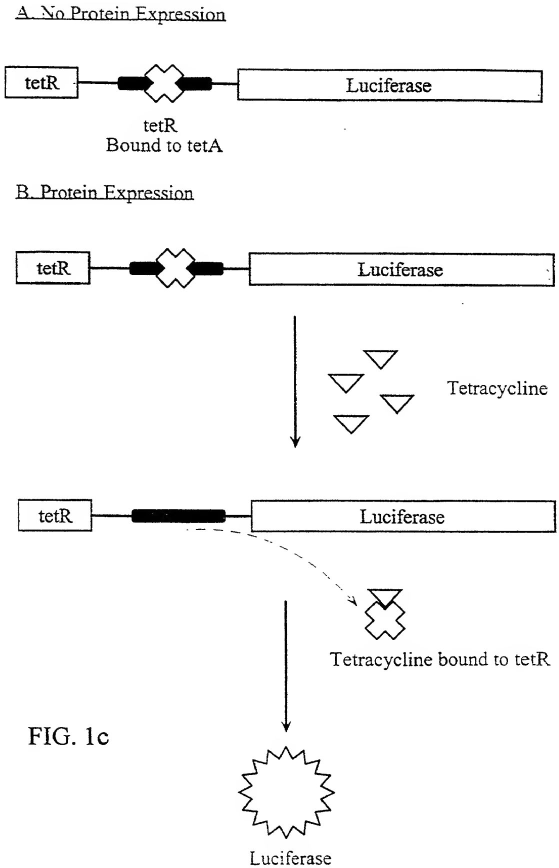
A. No Protein Expression
tetR
Luciferase
tetR
Bound to tetA
B. Protein Expression
tetR
Luciferase
Tetracycline
tetR
Luciferase
Tetracycline bound to tetR
Luciferase

FIG. 1c

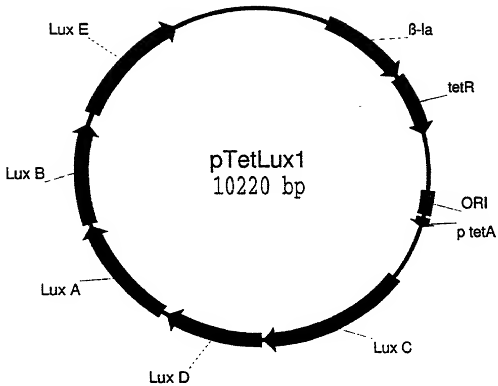
Lux E
ß-la
tetR
pTetLux1
10220 bp
Lux B
ORI
p tetA
Lux A
Lux C
Lux D

FIG. 2

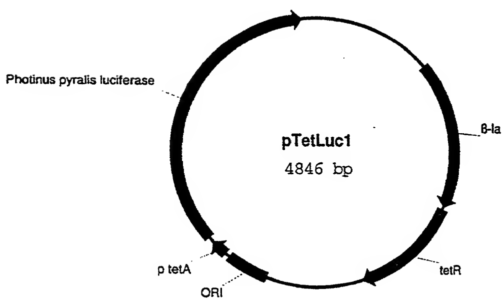
Photinus pyralis luciferase
pTetLuc1
4846 bp
ß-la
p tetA
tetR
ORI

FIG. 3

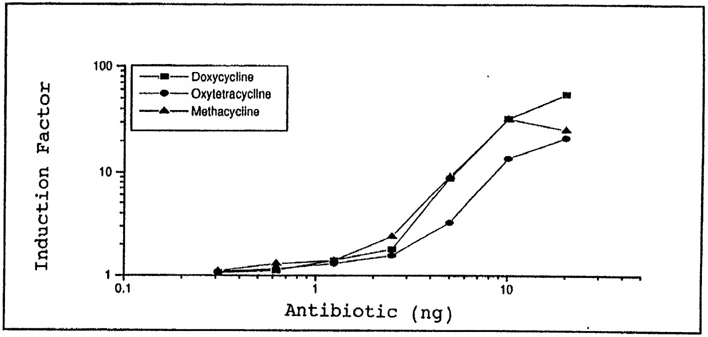
Induction Factor
100
10
1
0.1
1
10
Antibiotic (ng)
Doxycycline
Oxytetracycline
Methacycline

FIG. 4a

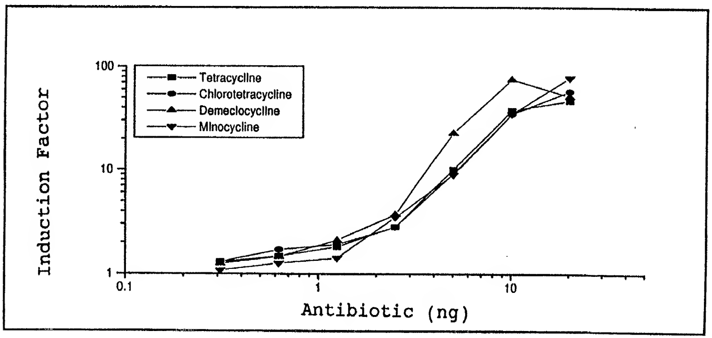
Induction Factor
100
10
1
0.1
1
10
Antibiotic (ng)
Tetracycline
Chlorotetracycline
Demeclocycline
Minocycline

FIG. 4b

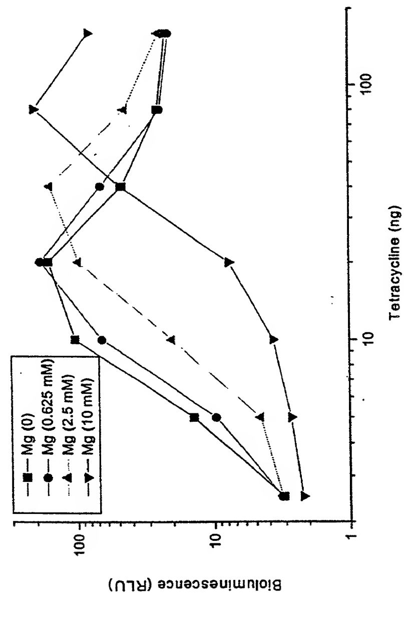
Mg (0)
Mg (0.625 mM)
Mg (2.5 mM)
Mg (10 mM)
Bioluminescence (RLU)
1
10
100
Tetracycline (ng)
10
100

FIG. 5

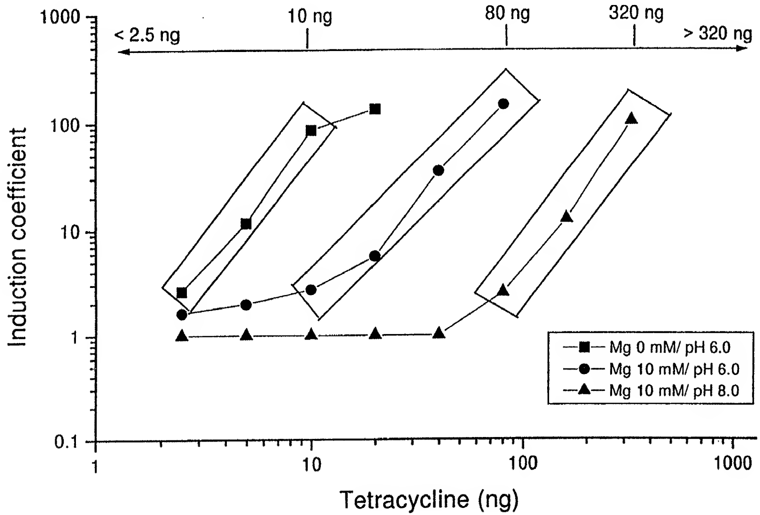
1000
100
10
1
0.1
Induction coefficient
< 2.5 ng
10 ng
80 ng
320 ng
> 320 ng
Mg 0 mM/ pH 6.0
Mg 10 mM/ pH 6.0
Mg 10 mM/ pH 8.0
1
10
100
1000
Tetracycline (ng)

FIG. 6

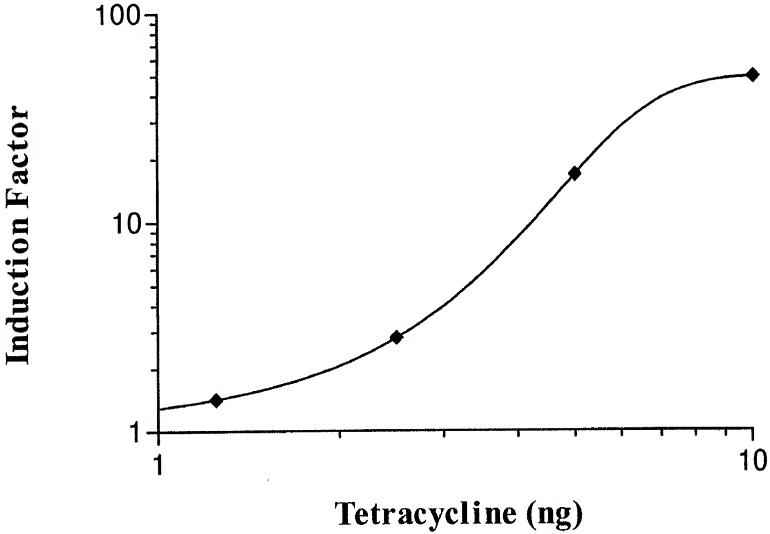

FIG. 7

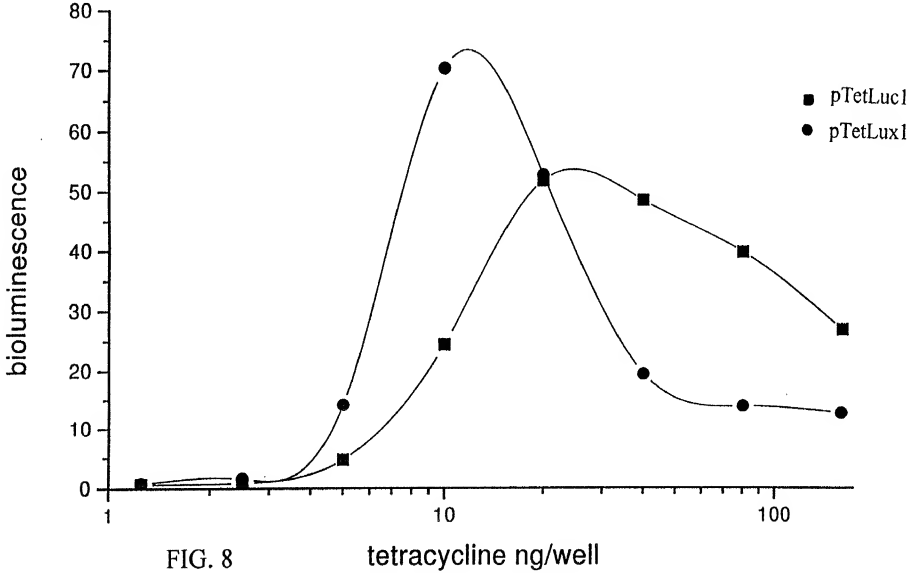
bioluminescence
0
10
20
30
40
50
60
70
80
1
10
100
tetracycline ng/well
pTetLuc1
pTetLux1

FIG. 8

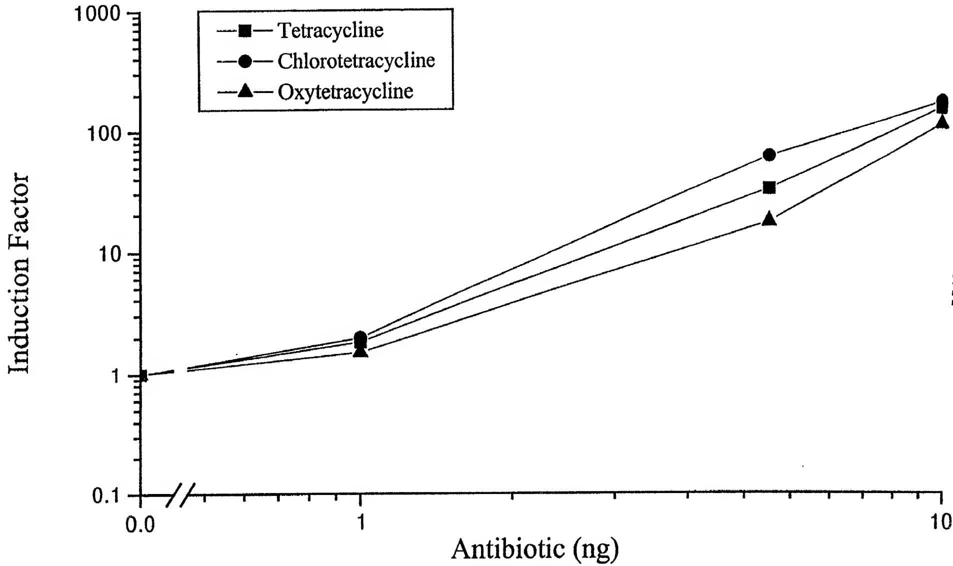
Induction Factor
1000
100
10
1
0.1
0.0
1
10
Antibiotic (ng)
Tetracycline
Chlorotetracycline
Oxytetracycline

FIG. 9

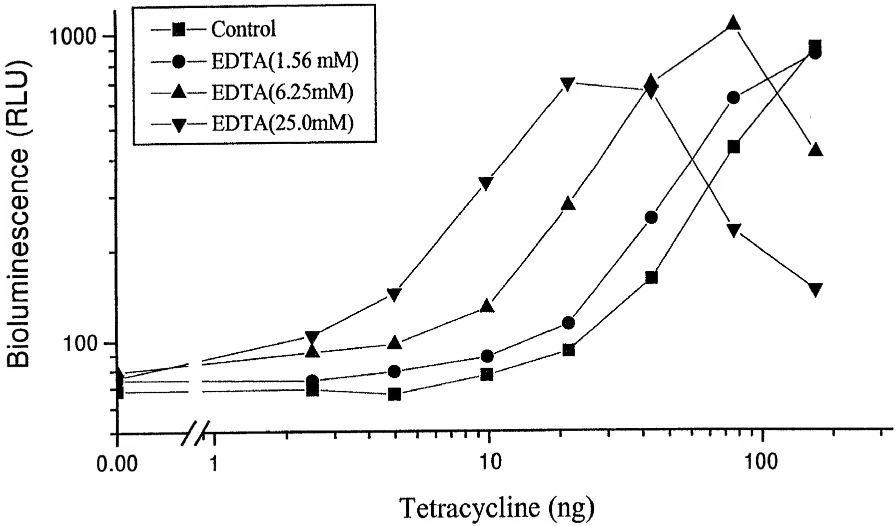
Bioluminescence (RLU)
1000
100
Control
EDTA(1.56 mM)
EDTA(6.25mM)
EDTA(25.0mM)
0.00
1
10
100
Tetracycline (ng)

FIG.10

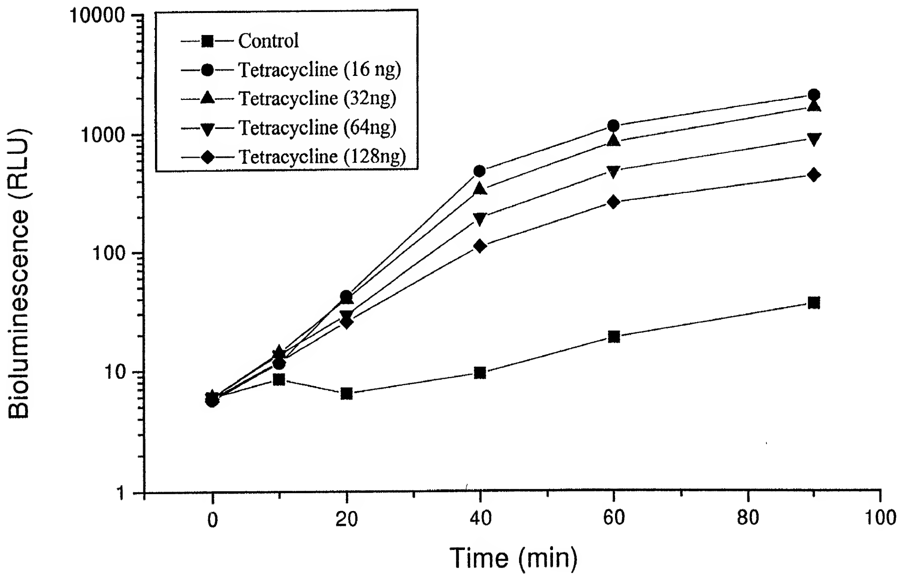
Bioluminescence (RLU)
10000
1000
100
10
1
0
20
40
60
80
100
Time (min)
Control
Tetracycline (16 ng)
Tetracycline (32ng)
Tetracycline (64ng)
Tetracycline (128ng)

FIG.11

Attorney Docket No. ______________

Declaration and Power of Attorney
For Patent Application
(Sole/Joint)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought, on the invention entitled "Tetracycline assay method",

the specification of which *(Check One)*

__ is attached hereto.

X was filed on November 11, 1998 as
[ ] Application Serial No. ______________
[X] International Application No. PCT/FI98/00873
and was amended on ______________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

(Number)	*(Country)*	*(Day/Month/Year Filed)*	**Priority Claimed**
974235	Finland	14/11/1997	Yes: X No: ___
			Yes: ___ No: ___
			Yes: ___ No: ___

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose

material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status)
(Application Serial No.)	(Filing Date)	(Status)

I or we hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to ROTHWELL, FIGG, ERNST & KURZ, P.C., 555 13th Street, N.W., Washington, D.C 20004

G. Franklin Rothwell, Reg. No. 18,125	Michael G. Sullivan, Reg. No. 35,377
E. Anthony Figg, Reg. No. 27,195	Mark I. Bowditch, Reg. No. 40,315
Barbara G. Ernst, Reg. No. 30,377	Robert J. Jondle, Reg. No. 33,915
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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